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(71) Applicants *(for all designated States except US)*: CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED [GB/GB]; Cambridge House, 6–10 Cambridge Terrace, Regent's Park, London NW1 4JL (GB). THE BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NEIDLE, Stephen [GB/GB]; University of London, The Institute of Cancer Research, CRC Biomolecular Structure Unit, Cotswold Road, Sutton, Surrey SM2 5NG (GB). JENKINS, Terence, Charles [GB/GB]; University of Greenwich, School of Chemistry & Life Sciences, Wellington Street, Woolwich SE18 6PF (GB). HURLEY, Laurence, Harold [US/US]; University of Texas System, 201 West 7th Street, Austin, TX 78701 (US). PERRY, Philip, John [GB/GB]; University of London, The institute of Cancer Research, CRC Biomolecular Structure Unit, Cotswold Road, Sutton, Surrey SM2 5NG (GB).

(74) Agents: GOLDIN, Douglas, Michael et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

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(57) Abstract

Novel 1,4– and 2,6–substituted anthracene–9,10–diones ("9,10–anthraquinones"). The use of the novel compounds and known 1,4– and 2,6–substituted anthracene–9,10–diones ("9,10–anthraquinones") in the inhibition of telomerase activity and/or their use in the treatment of cancer.

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ANTHRAQUINONES WITH BIOLOGICAL ACTIVITY

The present invention relates to anthraquinone compounds, processes for their production and their use as inhibitors of telomerase.

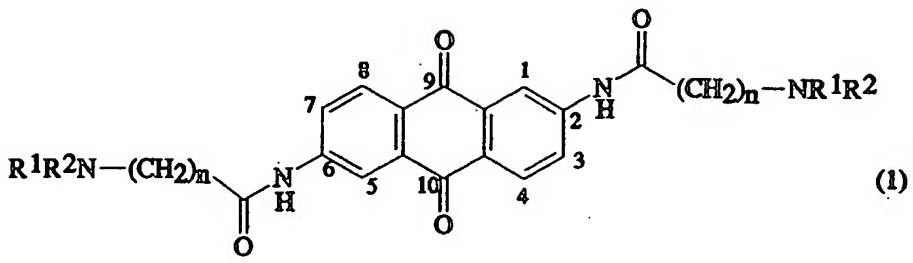
Eukaryotic cells contain chromosomes which divide and replicate during cell division. The ends of the chromosomes - telomeres - comprise tandem repeats of simple DNA sequences. These telomeric repeat sequences are essential for replication although in most normal cell types the length of the telomere is shortened by the process of replication. Cell senescence is closely correlated with a progressive reduction in the number of these repeats, and it is believed that senescence may be caused by a failure to maintain the length of the telomeres.

Further evidence for this can be found in the fact that germ cells and immortalized cancer cells do not suffer the same reduction in the length of telomeres during cell division, due to the activity in these cells of the telomerase enzyme. This enzyme is a ribonuclear protein containing an RNA template for the synthesis of the tandem repeat units of the telomeres.

Almost all tumor cells have shortened telomeres, which are maintained at constant length and which are associated with chromosome instability and cell immortalization. The enzyme telomerase adds the telomeric repeat sequences onto telomere ends, ensuring the net maintenance of telomere length in tumor cells resulting in successive rounds of cell division (D. Sun *et al*, J.Med. Chem., 40:2113-2116 (1997)).

Telomerase activity can be found in about 85 to 90% of human tumour cell types, including leukaemias, small cell and non-small cell lung cancer, myeloma, lymphoma, prostate, colon, head and neck, melanoma, Hepatocellular carcinoma, bladder, ovarian, breast and gastric cancers.

WO91/00265 (Neidle *et al*) discloses anti-cancer agents which are anthraquinones of formula (1):

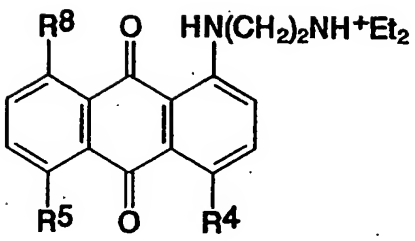


(1)

in which n is 1, 2 or 3; and R^1 and R^2 are each independently an ethyl, hydroxyethyl or hydroxymethyl group; or R^1 and R^2, together with the nitrogen atom to which they are attached, form a cyclic group which is a 1-piperidino, 2- or 4-(2-hydroxyethyl)-1-piperidino, 2-hydroxymethyl-1-piperidino, 4-(2-hydroxyethyl)- or 4-methyl-1-piperazino, or 4-morpholino group; or a pharmaceutically acceptable salt thereof.

Agbandje *et al*, J. Med. Chem., 35: 1418-1429 (1992) describes 9,10-anthraquinones which are examples of the compounds of formula (1) above and allegedly have potential as anticancer agents.

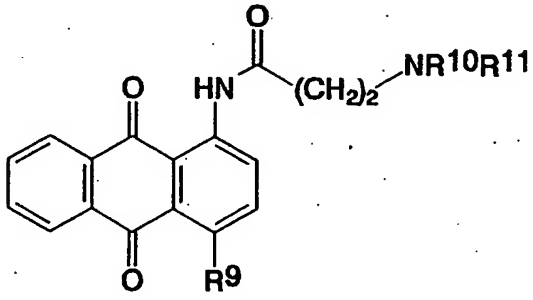
Tanious *et al*, Biochem., 31: 11632-11640 (1992) describes DNA-binding agents which are examples of the 9,10-anthraquinones of formula (1) above and four 9,10-anthraquinones of formula (2):



(2)

in which firstly R^4, R^5 and R^8 are all hydrogen, or in the other three compounds one of R^4, R^5 and R^8 is $NH(CH_2)_2NH^+Et_2$ while the other two of R^4, R^5 and R^8 are hydrogen.

Collier and Neidle, J. Med. Chem., 31: 847-857 (1988) describes a series of 1- and 1,4-substituted amidoanthraquinones of formula (3) that bind to DNA (and thus can be cytotoxic).



(3)

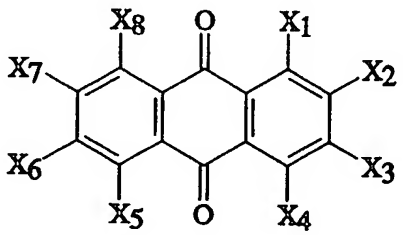
in which R^{10} and R^{11} are each independently an ethyl group; or R^{10} and R^{11} together with the nitrogen atom to which they are attached represent a heterocyclic group which is a 1-piperidino, 4-hydroxypropyl-1-piperazino or 2-hydroxyethyl-1-piperidino group; R^9 is hydrogen or $NHCO(CH_2)_2NR^{10}R^{11}$, in which R^{10} and R^{11} are as defined above.

Some of the compounds of formulae (1), (2) and (3) above have been proposed as anti-cancer agents although to date none have been developed beyond *in vitro* studies because they have been found to have only moderate

activity in conventional *in vivo* tumour cell lines, and moderate activity against animal models for cancer (Agbandje, M. PhD thesis, University of London, 1989).

However we have investigated compounds within the scope of formulae (1) and (3) above and surprisingly found that these compounds are inhibitors of telomerase. These findings have enabled us to develop novel compounds which also have this activity. The anthraquinones of formula I and II of the present invention have extended planar aromatic groups suitable for intercalation, together with at least one side-chain, each having a planar group at one end such as an amide which is itself attached to the aromatic chromophore, together with a neutral amine or cationic group at the other end. The compounds of the present invention preferably have two side-chains.

Thus in a first aspect the present invention provides novel anthraquinones of the formula (I) and pharmaceutically acceptable acid addition salts and quaternary ammonium salts thereof:



(I)

in which: each of X_1 and X_4, which are the same or different, is $HNCO(CH_2)_nNR^1R^2$, wherein each of R^1 and R^2, which are the same or different, is an unsubstituted or substituted alkyl group or R^1 and R^2 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted heterocyclic group, and n is an integer of from 1 to 6;

each of X_2, X_3, X_5, X_6, X_7 and X_8, which are the same or

different, is H, an unsubstituted or substituted alkyl group or halogen;

provided that:

when X_1 and X_4 are both $HNCO(CH_2)_nNR^1R^2$, and X_2, X_3, X_5, X_6, X_7 and X_8 are each H and n is 2, either R^1 and R^2 do not both represent ethyl, or R^1 and R^2 together with the nitrogen atom to which they are attached do not represent 1-piperidino or 2-hydroxymethyl-piperidino.

Preferably, both groups R^1 are the same and both groups R^2 are the same.

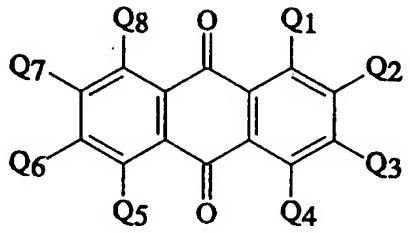
Preferably, each of X_2, X_3, X_5, X_6 X_7 and X_8 is hydrogen. Preferably R^1 and R^2 are methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. More preferably R^1 and R^2 are the same or R^1 and R^2 together with the nitrogen atom to which they are attached form a heterocyclic group. Preferably the heterocyclic group is a 4 to 8 membered ring, for example a hexamethyleneimino, heptamethyleneimino, azetidino, pyrrolidino, morpholino or 1-piperidino group which is unsubstituted or substituted with at least one C_1-C_6 alkyl group and/or at least one hydroxy group. More preferably, the heterocyclic group is an unsubstituted hexamethyleneimino, heptamethyleneimino, azetidino, pyrrolidino, morpholino or piperidino group or a 2-hydroxymethyl-piperidino group. The heterocyclic group may be a bicyclic ring such as an azabicyclic octano ring, for example 1,3,3-trimethyl-6-azabicyclo[3.2.1] octano. Preferably n is an integer of from 1 to 4, for example 1, 2 or 3, most preferably 2.

If R^1 and R^2 are not the same, preferably at least one of R^1 and R^2 is hydrogen or C_1 to C_6 alkyl. Most preferably at least one of R^1 and R^2 is hydrogen, methyl or ethyl. For example, R^1 is 2-hydroxyethyl and R^2 is ethyl, R^1 is methyl and R^2 is hydrogen, R^1 is $CH_2CH_2N(C_2H_5)_2$ and R^2 is methyl or R^1 is $CH_2CH_2NHCH_3$ and R^2 is methyl.

A substituted or unsubstituted alkyl group typically

contains 1 to 6 carbon atoms, for example methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. Suitable substituents include OH, halogen, NH_2, $N(C_1\text{-}C_6$ alkyl)H and $N(C_1\text{-}C_6$ alkyl)$_2$. Typically a substituted alkyl group has from 1 to 6 substituents. Preferred substituted alkyl groups include trifluoromethyl, $N(C_1\text{-}C_6$ alkyl)H such as $N(CH_3)H$ and $N(C_1\text{-}C_6$ alkyl)$_2$ such as $N(C_2H_5)_2$. Halogen is typically F, Cl, Br or I, preferably F.

In a second aspect the present invention provides compounds of the formula (II) and pharmaceutically acceptable acid addition salts or quaternary ammonium salts thereof:



in which: each of Q_2 and Q_6, which are the same or different, is $HNCO(CH_2)_nNR^3R^4$, wherein each of R^3 and R^4, which are the same or different, is an unsubstituted or substituted alkyl group or R^3 and R^4 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted heterocyclic group, and n is an integer of from 1 to 6;

each of Q_1, Q_3, Q_4, Q_5, Q_7, and Q_8, which are the same or different is H, OH, an amino or substituted amino group, an unsubstituted or substituted alkyl group or halogen;

provided that:

when Q_2 and Q_6 are both $HNCO(CH_2)_nNR^3R^4$, and Q_1, Q_3, Q_4, Q_5, Q_7 and Q_8 are each H and n is 1, 2 or 3, either NR^3R^4 is not $N(CH_2CH_3)_2$ or $N(CH_2CH_2OH)_2$ or R^3 and R^4 together with

the nitrogen atom to which they are attached do not represent piperidino, morpholino, 4-methylpiperazino, 2-hydroxymethyl-piperidino , 2-hydroxyethyl-piperazino or 4-hydroxyethyl-piperidino.

Preferably, both groups R^3 are the same and both groups R^4 are the same.

Preferably, each of Q_1, Q_3, Q_4, Q_5 Q_7 and Q_8 is hydrogen and Q_2 and Q_6 are $HNCO(CH_2)_nNR^3R^4$. Preferably R^3 and R^4 are methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl or hydroxyethyl. More preferably R^3 and R^4 are the same or R^3 and R^4 together with the nitrogen atom to which they are attached form a heterocyclic group. Preferably, the heterocyclic group is a 4 to 8 membered ring, for example a hexamethyleneimino, heptamethyleneimino, azetidino, pyrrolidino, morpholino or piperidino group which is unsubstituted or substituted with at least one C_1-C_6 alkyl group and/or at least one hydroxy group. More preferably, the heterocyclic group is an unsubstituted hexamethyleneimino, heptamethyleneimino, azetidino, pyrrolidino, morpholino or piperidino group or a hydroxymethyl-piperidino group. The heterocyclic group may be a bicyclic ring such as an azabicyclic octano ring, for example 1,3,3-trimethyl-6-azabicyclo[3.2.1]octano. Preferably n is an integer of from 1 to 4, for example 1, 2 or 3, most preferably 2.

If R^3 and R^4 are not the same, preferably at least one of R^3 and R^4 is hydrogen or C_1 to C_6 alkyl. Most preferably at least one of R^3 and R^4 is hydrogen, methyl or ethyl. For example, R^3 is 2-hydroxyethyl and R^4 is ethyl, R^3 is methyl and R^4 is hydrogen, R^3 is $CH_2CH_2N(C_2H_5)_2$ and R^4 is methyl or R^3 is $CH_2CH_2NHCH_3$ and R^4 is methyl.

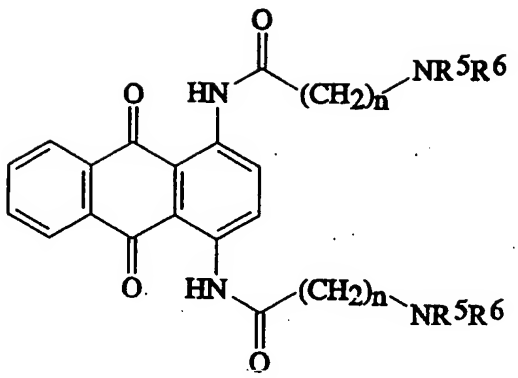
A substituted or unsubstituted alkyl group typically contains 1 to 6 carbon atoms, for example methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. Suitable substituents include OH, halogen, NH_2, $N(C_1$-C_6 alkyl)H, and

$N(C_1\text{-}C_6$ alkyl$)_2$. Typically a substituted alkyl group has from 1 to 6 substitutents. Preferred substituted alkyl groups include trifluoromethyl, $N(C_1\text{-}C_6$ alkyl$)H$ such as $N(CH_3)H$ and $N(C_1\text{-}C_6$ alkyl$)$ such as $N(C_2H_5)_2$. Halogen is typically F, Cl, Br or I, preferably F.

An amino group is a $-NH_2$ group and a substituted amino group is typically a -NHR or $-NR_2$ in which the two groups R may be the same or different. Typically R is a substituted or unsubstituted alkyl group and preferably contains 1 to 6 carbon atoms, for example methyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl. Suitable substituents include OH and/or halogen. Typically a substituted alkyl group has from 1 to 6 substituents.

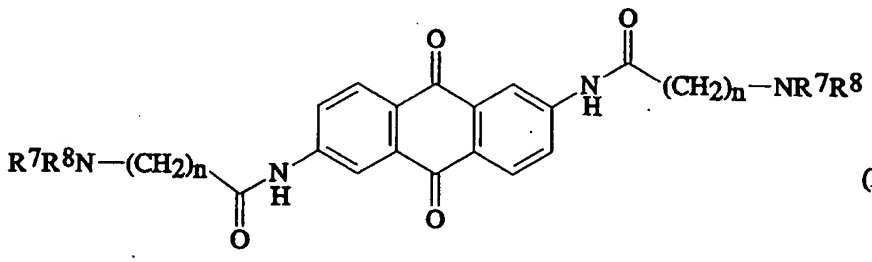
Preferably, the anthraquinones of formulae (I) and (II) are symmetrical. For example, in anthraquinones of formula (I) the groups X_1 and X_4, X_2 and X_3, X_5 and X_8 and X_6 and X_7 are the same and in anthraquinones of formula (II) the groups Q_1 and Q_5, Q_2 and Q_6, Q_3 and Q_7 and Q_4 and Q_8 are the same.

The invention also provides a method for inhibiting the activity of telomerase in a cell in which telomerase is active which comprises adding to the cell or its environment an effective amount of an anthraquinone of formula (I), formula (II), formula (III):



(III)

in which: R^5 and R^6 are each independently ethyl; or R^5 and R^6 together with the nitrogen atom to which they are attached represent a heterocyclic group which is a 2-hydroxymethyl-1-piperidino or 1-piperidino group; and n is 2; or formula (IV):



(IV)

in which: each R^7 group is the same, each R^8 group is the same and R^7 and R^8 are each independently an ethyl or 2-hydroxyethyl group; or R^7 and R^8 together with the nitrogen atom to which they are attached represent a heterocyclic group which is a 1-piperidino, 2-hydroxymethyl-1-piperidino, or 4- or 2-(2-hydroxyethyl)-1-piperidino group; and n is 1, 2 or 3; or a pharmaceutically

acceptable acid addition salt or quaternary ammonium salt thereof.

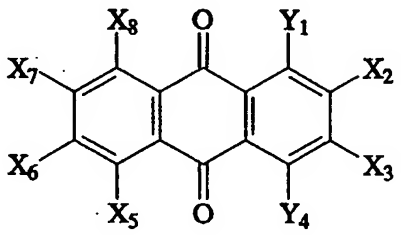
The invention also provides anthraquinones of the formula (I) or (II) or pharmaceutical compositions thereof for use in the treatment of the human or animal body, particularly for the treatment of cancers.

The invention further provides the use of anthraquinones of formula (I), (II), (III) or (IV) for the manufacture of a medicament for inhibiting the activity of telomerase and/or for treating cancer.

The invention further provides a process for the production of an anthraquinone of formula (I) or (II) as defined above which comprises aminolysis of a mono- or bis-(ω-haloalkylcarboxamido)-substituted anthraquinone or, alternatively, acylation of a mono- or diaminoanthraquinone with a ω-aminoalkylalkanoic acid or a derived acylating derivative.

Thus, the present invention provides a process for the production of an anthraquinone of formula (I) or (II), which process comprises:

i) reacting an intermediate of formula (B):



(B)

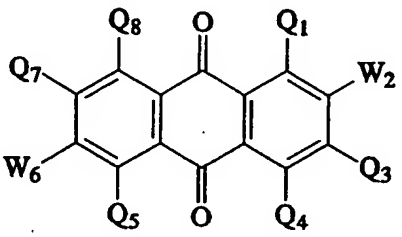
in which: each of Y_1 and Y_4, which are the same or different, is $HNCO(CH_2)_nZ$, wherein Z is a leaving group and n is an integer of from 1 to 6, and X_2, X_3, X_5, X_6, X_7 and X_8 are as defined above for the anthraquinones of formula (I);

with the compound of formula (C):

R^1R^2NH (C)

wherein R^1 and R^2 are as defined above for the anthraquinones of formula (I); or

ii) reacting a intermediate of formula (A):



(A)

in which: each of W_2 and W_6, which are the same or different, is $HCO(CH_2)_nZ$, an unsubstituted or substituted alkyl group or halogen, wherein Z is a leaving group and n is an integer of from 1 to 6, and Q_1, Q_3, Q_4, Q_5, Q_7 and Q_8 are as defined above for the anthraquinones of formula (II);

with a compound of formula (D):

R^3R^4NH (D)

wherein R^3 and R^4 are as defined above for the anthraquinone of formula (II).

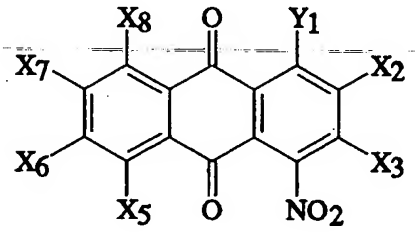
Suitable leaving groups, Z, include halogen, for example F, Cl, Br, I and sulfonate esters of formula $-OSO_2R$ where R is C_{1-6} alkyl, aralkyl or aryl, or other functionalities which can be replaced by aminolysis. Chlorine is a particularly preferred leaving group.

The intermediate of formula (B) can be obtained using the method described in Collier and Neidle, J. Med. Chem., 31: 847-857 (1988). The intermediate of formula (A) can be obtained using the method described in Agbandje *et al.*, J. Med. Chem. 35: 1418-1429 (1992).Further suitable intermediates can be readily obtained using established synthetic procedures for ring-substituted anthraquinones, as described in Bayer, Methoden der Organischen Chemie

7/3c, Verlag, page 111 (1974), and in Zagotto *et al.*, Bioorg. Med. Chem. Lett. 2: 659 (1992). Other anthraquinone derivatives for use as starting materials are available from published synthetic methods, or by ready adaption thereof, or from commercial sources.

The present invention also provides a process for producing anthraquinones of formula (I) in which the two groups R^1 are not the same and/or the two groups R^2 are not the same, which process comprises:

(i) reacting an intermediate of formula (B'):



(B')

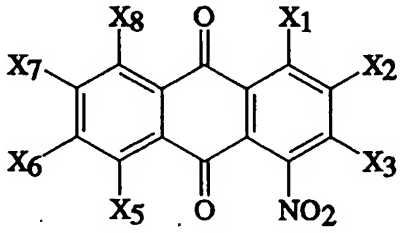
in which:

Y_1 is $HNCO(CH_2)_nZ$, wherein Z is a leaving group and n is an integer of from 1 to 6, and X_2, X_3, X_5, X_6, X_7 and X_8 are as defined above;

with a compound of formula (C)

$$R^1R^2NH \qquad (C)$$

wherein R^1 and R^2 are as defined above, to give a compound of formula (E):

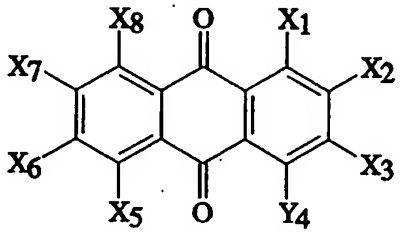


(E)

wherein X_1, is as defined above;

(ii) converting the NO_2 group to an NH_2 group;

(iii) reacting the product of step (ii) with $Z(CH_2)_nCOZ$ wherein Z is a leaving group and n is an integer of from 1 to 6, to give a product of formula (F):



(F)

in which Y_4 is $HNCO(CH_2)_nZ$;

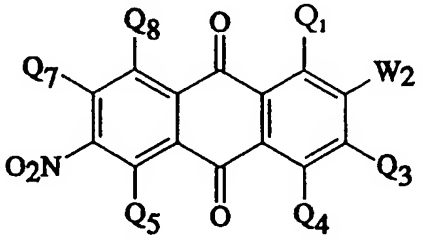
(iv) reacting the product of step (iii) with a compound of formula (C'):

$$R^{1'}R^{2'}NH \qquad (C')$$

wherein $R^{1'}$ and $R^{2'}$ have the same definition as R^1 and R^2 as defined above, with the proviso that the compound of formula (C^1) is not identical to the compound of formula (C) used in step (i), to give a compound of formula (I).

The present invention also provides a process for producing anthraquinones of formula (II) in which the two groups R^3 are not the same and/or the two groups R^4 are not the same, which process comprises:

(i) reacting an intermediate of formula (A'):



(A')

in which:

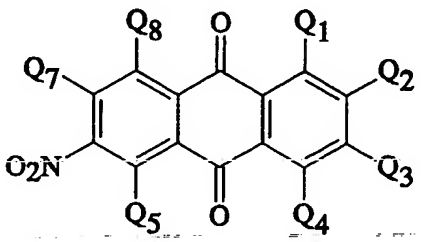
W_2 is $HNCO(CH_2)_nZ$, wherein Z is a leaving group and n

is an integer of from 1 to 6, and Q_1, Q_3, Q_4, Q_5, Q_7 and Q_8 are as defined above;

with a compound of formula (D)

$$R^3R^4NH \qquad (D)$$

wherein R^3 and R^4 are as defined above, to give a compound of formula (G):

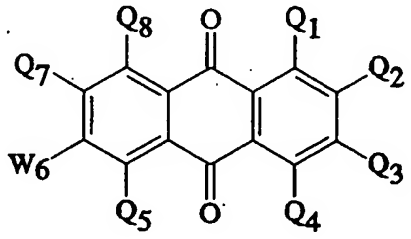


(G)

wherein Q_2 is as defined above;

(ii) converting the NO_2 group to an NH_2 group;

(iii) reating the product of step (ii) with $Z(CH_2)_nCOZ$ wherein Z is a leaving group and n is an integer of from 1 to 6 to give a product of formula (H):



(H)

in which W_6 is $HNCO(CH_2)_nZ$;

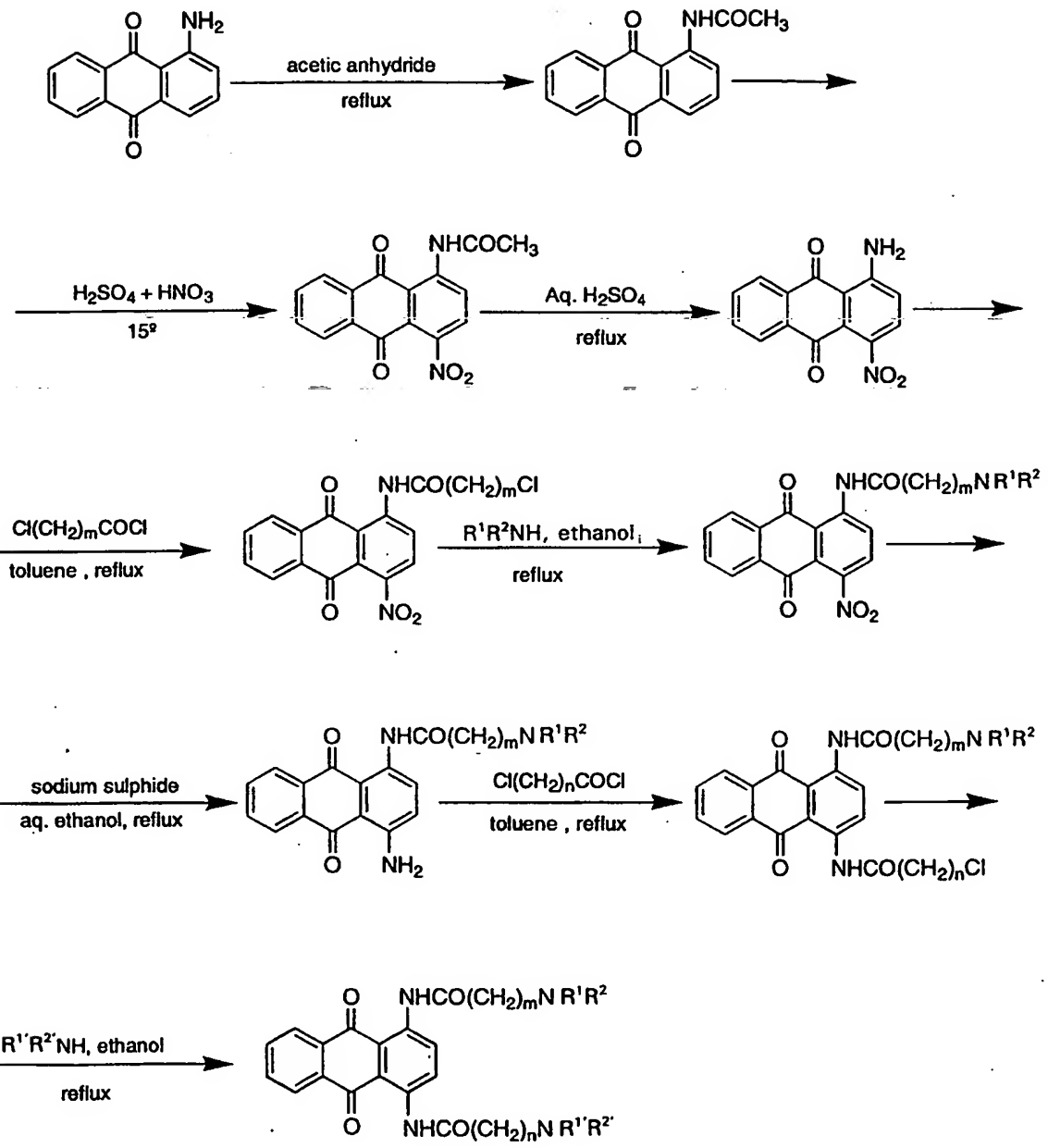
(iv) reacting the product of step (iii) with a compound of formula (D'):

$$R^{3'}R^{4'}NH \qquad (D')$$

wherein $R^{3'}$ and $R^{4'}$ have the same definition as R^3 and R^4 as defined above, with the proviso that the compound of formula (D') is not identical to the compound of formula (D) used in step (i), to give a compound of formula (II).

Anthraquinones of formula (I) in which the two groups R^1 are not the same and/or the two groups R^2 are not the same may be produced in accordance with the following reaction scheme.

5


NH2
acetic anhydride
reflux
NHCOCH3
H2SO4 + HNO3
15º
NO2
Aq. H2SO4
reflux
Cl(CH2)mCOCl
toluene , reflux
NHCO(CH2)mCl
R1R2NH, ethanol
NHCO(CH2)mN R1R2
sodium sulphide
aq. ethanol, reflux
Cl(CH2)nCOCl
NHCO(CH2)nCl
R1'R2'NH, ethanol
NHCO(CH2)nN R1'R2'

wherein the definition of $R^{1'}$ and $R^{2'}$ is the same as that for R^1 and R^2 above (with the proviso that $R^{1'}$ is not the same as R^1 and/or $R^{2'}$ is not the same as R^2) and m has the same meaning as n.

The skilled reader will appreciate that anthraquinones of formula (II) in which the two groups R^3 are not the same and/or the two groups R^4 are not the same may be produced using an analogous reaction scheme.

The invention provides a process for the production of a salt of an anthraquinone of any of formulae (I) to (IV) as defined above by subsequent alkylation treatment of a precursor compound of any of formulae (I) to (IV), preferably with an alkyl halide or aralkyl halide, to form the corresponding quaternary ammonium halide salt.

Physiologically acceptable salts according to the invention which may be conveniently used include physiologically acceptable acid addition salts, including the hydrochloride, acetate, maleate and, in particular, quaternary (eg methyl or ethyl iodide) salts. Preferred quaternary salts of compounds of formula (I) or (II) include those in which $-N^+R^1R^2R^9X^-$ or $-N^+R^3R^4R^9X^-$ have the same NR^1R^2 or NR^3R^4 substituent groups and R^9 is $-CH_3$ or $-CH_2CH_3$ and X^- is a iodide or physiologically acceptable anion.

Acid addition salts according to the invention include mono- and di-carboxylic acids in which the non-carbonyl moiety of the carboxylate grouping is selected from straight or branched chain alkyl (e.g. methyl, n-propyl, n-butyl or t-butyl); cyclic alkyl (e.g. cyclohexyl); alkoxyalkyl (e.g. methoxymethyl), carboxyalkyl (e.g. carboxyethyl), aralkyl (e.g. benzyl), aryloxyalkyl (e.g. phenoxymethyl), aryl (e.g. phenyl optionally substituted by halogen, C_{1-4} alkyl or C_{1-4} alkoxy or amino); sulfonic acids such as alkyl- or aralkyl-sulfonate (e.g. methanesulfonate); mono- or di-phosphoric

acids which may or may not be blocked, amino acids (e.g. L-valine or L-isoleucine) and nitrates. With regard to these acid components, unless otherwise specified, any alkyl moieties present in such acids preferably contain 1 to 18 carbon atoms, particularly 1 to 4 carbon atoms, in the case of straight chain alkyl groups, or 3 to 7 carbon atoms in the case of branched or cyclic alkyl groups. Any aryl moiety present in such acids advantageously comprises a phenyl group.

Any reference herein to any of the above compounds of the invention also includes a reference to a physiologically acceptable salt thereof.

Particular anthraquinones of formula (I) include:

1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione (BSU-1062);

1,4-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione (BSU-1079);

1,4-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione (BSU-1084);

1,4-Bis[3-(pyrrolidino)propionamido]anthracene-9,10-dione (BSU-1074);

1,4-Bis[3-(4-morpholino)propionamido]anthracene-9,10-dione (BSU-1076);

1,4-Bis(4-piperidinobutyramido)anthracene-9,10-dione (BSU-9027).

Particular anthraquinones of formula (II) include:

2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione (BSU-1060);

2,6-Bis[2-(bis(2-hydroxyethyl)amino)acetamido]anthracene-9,10-dione (BSU-1065);

2,6-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione (BSU-1082);

2,6-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione (BSU-1085);

2,6-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-

dione (BSU-1078);
2,6-Bis[3-[(2-hydroxyethyl)ethylamino]propionamido] anthracene-9,10-dione (BSU-6001);
2,6-Bis[3-(hexamethyleneimino)propionamido]anthracene-9,10-dione (BSU-9080);
2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione (BSU-9081);
2,6-Bis[3-(*N,N*-diethyl-*N'*-methylethylenediamino) propionamido] anthracene-9,10-dione (BSU-9082);
2,6-Bis[3-(methylamino)propionamido]anthracene-9,10-dione (BSU-9083);
2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione (BSU-9084);
2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano) propionamido] anthracene-9,10-dione (BSU-9085);
2,6-Bis[3-(*N,N*-dimethylethylenediamino)propionamido] anthracene-9,10-dione (BSU-6004).

Particular pharmaceutically acceptable salts of the anthraquinones of formula (I) include the corresponding hydrochloride salts, acetic acid salts, and:
1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1063);
1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione N,N'-diethiodide (BSU-1068);
1,4-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1080);
1,4-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1087);
1,4-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1075);
1,4-Bis[3-(4-morpholino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1077);
1,4-Bis(4-piperidinobutyramido)anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9031).

Particular pharmaceutically acceptable salts of the

anthraquinones of formula (II) include the corresponding hydrochloride salts, acetic acid salts, and:
2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1061);
2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione N,N'-diethiodide (BSU-1067);
2,6-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1083);
2,6-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1086);
2,6-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1081);
2,6-Bis[3-[(2-hydroxyethyl)ethylamino]propionamido]anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-6002);
2,6-Bis[3-(hexamethyleneimino)propionamido]anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9087);
2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9088);
2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9089);
2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano)propionamido] anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9091);
2,6-Bis[3-(*N,N*-diethyl-*N'*-methylethylenediamino)propionamido] anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9097);
2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione maleate salt (BSU-9086);
2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione maleate salt (BSU-9090);
2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano)propionamido] anthracene-9,10-dione maleate salt (BSU-9092);
2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione maleate salt (BSU-9093);

2,6-Bis[3-(*N*,*N*-diethyl-*N'*-methylethylenediamino)propionamido] anthracene-9,10-dione maleate salt (BSU-9094).

Particular anthraquinones of formula (III) include:
1,4-Bis[3-(2-hydroxymethyl-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1057);
1,4-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione (BSU-1070);
1,4-Bis[3-(2-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1064);
1,4-Bis[3-(1-piperidino)propionamido]anthracene-9,10-dione (BSU-1071).

Particular anthraquinones of formula (IV) include:
2,6-Bis[3-(2-hydroxymethyl-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1040);
2,6-Bis[3-(2-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1035);
2,6-Bis[3-(4-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1038);
2,6-Bis[3-(bis(2-hydroxyethyl)amino)propionamido]anthracene-9,10-dione (BSU-1041).

Particular quaternary ammonium salts of the anthraquinones of formula (III) include:
1,4-Bis[3-(2-hydroxymethyl-1-piperidino)propionamido] anthracene-9,10-dione N,N'-dimethiodide (BSU-1058);
1,4-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1073);
1,4-Bis[3-(1-piperidino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1072).

Particular pharmaceutically acceptable acid addition salts of the anthraquinones of formula (IV) include:
2,6-Bis(3-(1-piperidino)propionamido)anthracene-9,10-dione diacetate (BSU-1021);
2,6-Bis(2-(4-morpholino)acetamido)anthracene-9,10-dione

diacetate (BSU-1022);
2,6-Bis(2-diethylaminoacetamido)anthracene-9,10-dione diacetate (BSU-1024);
2,6-Bis(3-(4-morpholino)propionamido)anthracene-9,10-dione diacetate (BSU-1028); and
2,6-Bis(3-(4-(2-hydroxyethyl)-1-piperazino)-propionamido) anthracene-9,10-dione diacetate (BSU-1043).

Particular quaternary ammonium salts of the anthraquinones of formula (IV) include:
2,6-Bis[3-(2-(2-hydroxymethyl)-1-piperidino)propionamido] anthracene-9,10-dione N,N'-dimethiodide (BSU-1051);
2,6-Bis[3-(4-(2-hydroxyethyl-1-piperidino)propionamido] anthracene-9,10-dione N,N'-dimethiodide (BSU-1050);
2,6-Bis[3-bis(2-hydroxyethyl)amino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1052).

In addition to the above, compounds of formulae (I), (II), (III) and (IV) which are at least as active as 2,6-bis[3-(piperidino)propionamido]anthracene-9,10-dione acetate (BSU-1021) in the *in vitro* TRAP assay of Biological Assay are especially preferred.

The anthraquinones of formulae (I), (II), (III) and (IV) may be used *in vitro* or *in vivo* as telomerase inhibitors. For *in vitro* use, the compounds will be useful in the development and standardization of assays for telomerase and inhibitors thereof and in gene probe-based applications, or biological/molecular biological applications, for example microscopy. For example, in a preferred assay format described herein, telomerase is obtained from a partial purification of a mammalian cell extract. In order to standardize the activity of the assay or results for telomerase inhibitors using the assay, compounds of the invention may be used, e.g. those compounds which have already been used in previous assays of the same format using different cell extracts.

For *in vivo* use the compounds will be used in methods of treatment of uncontrolled cell proliferation, particularly cancers. Such cancers include leukaemias, small cell and non-small cell lung cancer, ovarian, breast, gastric, liver, cervical, colorectal, bladder, renal, stomach, brain, prostate, testicular, head and neck, skin and thyroid cancers, melanomas, non-Hodgkin's lymphoma, leukaemias, sarcomas and neuro-blastoma.

Because the inhibition of telomerase activity in a cell will not necessarily lead to cell death immediately the anthraquinones of formulae (I), (II), (III) and (IV) may be relatively slow acting. In view of this these compounds may be used as a single agent or in combination with other anti-cancer compounds, particularly cytotoxic compounds such as doxorubicin, cisplatin, or other anti-cancer treatments such as radiation, ADEPT (antibody-directed enzyme prodrug therapy), VDEPT (vector-directed enzyme prodrug therapy), and GDEPT (gene-directed enzyme prodrug therapy).

For example, a patient may first be treated with another anti-cancer compound or treatment which will destroy a substantial portion of the cancer. Alternatively, a patient may be treated simultaneously with another anti-cancer compound or treatment which will destroy a substantial portion of the cancer. In order to treat or control the regrowth of any residual primary tumour cells which may be resistant to the main therapy, anthraquinones of formulae (I), (II), (III) or (IV) may be administered to the patient over prolonged periods of time.

Such chronic administration may also be appropriate to prevent or treat secondary tumours in the event that metastatic spread of the primary tumour occurs. Anthraquinones of the formulae (I), (II), (III) or (IV) may also be used in conjunction with other compounds

designed to prevent or treat metastases, particularly matrix metalloproteinase inhibitors (MMIs).

Combined therapy with second compounds such as MMIs will be particularly advantageous since the second compound(s) can target a separate locus within the tumour cell, for example in the case of MMIs the enzymes responsible for invasion of the tumour cells. In this manner the tumour cells may be prevented from spreading for sufficient time such to inhibit telomerase activity for long enough to allow the cells to differentiate and/or senesce.

The anthraquinones of formulae (I), (II), (III) or (IV) may be administered to mammals including humans by any route appropriate to the condition to be treated, suitable routes including oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). It will be appreciated that the preferred route may vary with, for example, the condition of the recipient.

For each of the above-indicated utilities and indications the amount required of the individual active ingredients will depend upon a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician. In general, however, for each of these utilities and indications, a suitable, effective dose will be in the range 0.01 to 50 mg per kilogram body weight of recipient per day, preferably in the range 0.01 to 20 mg per kilogram body weight per day and most preferably in the range 0.01 to 10 mg per kilogram body weight per day (unless otherwise indicated all weights of active ingredient are calculated as the parent compound; for salts thereof the figures would be increased proportionately.)

The desired dose may if desired be presented as two, three, four or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 0.1 to 3000 mg, preferably 0.1 to 650 mg of active ingredient per unit dosage form.

Doses of compounds of the invention may be administered at sub-daily, or daily intervals, or less frequently, for example on alternate days, weekly or fortnightly. In general the doses will be the same as the above daily dose although higher doses, particularly when formulated to be released over a prolonged period of time, may be used.

While it is possible for the compounds to be administered alone it is preferable to present them as pharmaceutical formulations. The formulations of the present invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof.

The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with

liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent.

A capsule may be made by filling a loose or compressed powder on an appropriate filling machine, optionally with one or more additives. Examples of suitable additives include binders such as povidone; gelatin, lubricants, inert diluents and disintegrants as for tablets.

Compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound 1) in an optionally buffered, aqueous solution or 2) dissolved in an adhesive or 3) dispersed in a polymer. A suitable concentration of the active compound is about 1% to 35%, preferably about 3% to 15%.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection

solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

Where anthraquinones of the formulae (I), (II), (III) or (IV) are used in conjunction with second anti-cancer compounds, the active ingredient(s) and pharmacologically active agents may be administered together or separately and, when administered separately this may occur simultaneously or sequentially in any order. The amounts of the active ingredient(s) and pharmacologically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

The anthraquinones of formula (I) and (II) may be produced by various methods known in the art of organic chemistry in general. Starting materials are either known and readily available from commercial sources or may themselves be produced by known and conventional techniques.

The following examples illustrate the invention. For the purposes of clarity, the examples are presented in two sections; section A illustrates the synthesis of anthraquinones of formulae (I), (II), (III) and (IV) and salts thereof, and section B illustrates the biological assays of compounds of the invention.

Section A - Preparative Methods

Preparative method for anthraquinone free bases of formula (I) and acid addition salts thereof:

Example 1

1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione (BSU-1062)

(A) *Aminolysis of 1,4-bis(3-chloropropionamido)anthraquinone*

Dimethylamine (5.4 mL of 33% EtOH solution, 0.03 mol) was added during 15 min to a stirred, refluxing suspension of 1,4-bis(3-chloropropionamido)anthracene-9,10-dione (intermediate B; 1 g, 2.4 mmol; prepared by the published procedure of Collier & Neidle, *J. Med. Chem.* 1988, *31*, 847-857) in EtOH (50 mL). After reflux for 40 min, at which time TLC (silica gel; EtOH) indicated completion of reaction, the mixture was concentrated to 25 mL by solvent removal and chilled to 0-5 °C using an external ice-water bath. The solid that separated was removed by filtration and washed with dry ether to give the title product (1.02 g, 91%) as an amorphous brown solid, mp 167-168 °C.

(B) *Preparation of hydrochloric acid addition salt (General Procedure)*

Dry HCl gas is passed into a rapidly stirred solution or suspension of the aminoamide (1-2 mmol) in acetone cooled to 0-5 °C, until saturation is achieved. The solid which separates is collected by filtration, washed with dry ether (3 x 20 mL), and finally dried in vacuo (P_2O_5, <1 mm Hg) at 25 °C.

Treatment of the free base using this general procedure gave the dihydrochloride salt (95%) as an amorphous powder: mp >280 °C dec.; NMR δ 2.24 (s, 12H, NCH_3), 2.64 (br m, 8H, $COCH_2CH_2N$), 7.93 (m, 2H, H-6,7), 8.18 (m, 2H, H-5,8), 8.89 (s, 2H, H-2,3), and 12.27 (s, D_2O removes, 2H, CONH); IR (KBr) 3435 (NH), 3247, 2944, 1697

(C=O), 1636 (quinone C=O), 1592, and 1582 cm^{-1}; UV/vis [CH_3OH, λ (log ϵ)], 254 (5.77), 310 (5.05), and 463 (4.84) nm; MS (rel intensity) *m/z* 436 ([*M*]$^{\cdot+}$, 6), 391 ([*M*-C_2H_7N]$^{\cdot+}$, 7), 346 ([*M*-$C_4H_{14}N_2$]$^{\cdot+}$, 56), 292 ([*M*-$C_7H_{16}N_2O$]$^{\cdot+}$, 36), 238 ([*M*-$C_{10}H_{18}N_2O_2$]$^{\cdot+}$, 50), and 56 ([C_3H_4O]$^{\cdot+}$, 100). Anal. Found: C, 65.11; H, 6.30; N, 12.51%. $C_{24}H_{28}N_4O_4 \cdot 0.25H_2O$ requires C, 65.36; H, 6.51; N, 12.70%.

Example 2

1,4-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione (BSU-1079)

Dipropylamine (1.2 g, 0.012 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **B** (0.5 g, 1.2 mmol) in EtOH (40 mL). After 2 h of reflux, at which time TLC (silica gel; EtOH-CH_2Cl_2 1:1 v/v as eluent) indicated completion of reaction, the mixture was chilled to 0-5 °C and water (50 mL) was added. The precipitated solid was filtered, washed with water and dried in vacuo at 40 °C. The free base (0.58 g, 89%) was obtained as a red brown solid, mp 97-98 °C. The derived dihydrochloride salt was prepared, using the general procedure outlined above for BSU-1062, in the form of an amorphous powder: mp 212-213 °C. Anal. Found: C, 69.56; H, 8.02; N, 10.12%. $C_{32}H_{44}N_4O_4 \cdot 0.25H_2O$ requires C, 69.47; H, 8.11; N, 10.13%.

Example 3

1,4-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione (BSU-1084)

Dibutylamine (1.6 g, 0.012 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **B** (0.5 g, 1.2 mmol) in EtOH (40 mL). After 3 h reflux, at which time TLC (silica gel; EtOH-CH_2Cl_2 1:1 v/v as eluent) indicated completion of reaction, the solvent was removed and the solid washed with water,

filtered and dried in vacuo at 30 °C (P_2O_5). The free base product (0.63 g, 88%) was obtained as a red-brown solid, mp 51-52 °C. The dihydrochloride salt, mp 190-191 °C, was prepared from the free base using the general procedure described above for BSU-1062. Anal. Found: C, 70.87; H, 8.50; N, 9.16%. $C_{36}H_{52}N_4O_4 \cdot 0.25H_2O$ requires C, 70.96; H, 8.68; N, 9.19%.

Example 4

1,4-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione (BSU-1074)

Pyrrolidine (1.7 g, 0.024 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **B** (1 g, 2.4 mmol) in EtOH (60 mL). After reflux for 40 min, TLC (silica gel; EtOH-CH_2Cl_2 1:1 v/v as eluent) indicated completion of reaction. The mixture was chilled to 0-5 °C and water (100 mL) was added. The precipitated solid was filtered, washed with water and dried in vacuo at 40 °C. The title compound (1.16 g, 97%) was obtained as a red-brown solid, mp 163-164 °C. The corresponding dihydrochloride salt was prepared, using the general procedure outlined for BSU-1062 above, in the form of an amorphous powder: mp 264-265 °C dec.; NMR δ 1.72 (m, 8H, pyrr. H-3,4), 2.50 (m, 8H, pyrr. H-2,5), 2.65 (t, J = 6.6 Hz, 4H, $COCH_2CH_2N$), 2.80 (t, J = 6.6 Hz, 4H, $COCH_2CH_2N$), 7.94 (m, 2H, H-6,7), 8.18 (m, 2H, H-5,8), 8.89 (s, 2H, H-2,3), and 12.30 (s, D_2O removes, 2H, CONH); IR (KBr) 3410 (NH), 2961, 2798, 1697 (C=O), 1636 (quinone C=O), 1592, and 1583 cm^{-1}; UV/vis [CH_3OH, λ (log ϵ)], 253 (5.66), 312 (4.98), and 459 (4.82) nm; MS (rel intensity) *m/z* 488 ($[M]^{\cdot+}$, 3), 417 ($[M-C_4H_9N]^{\cdot+}$, 29), 346 ($[M-C_8H_{16}N_2]^{\cdot+}$, 72), 292 ($[M-C_{11}H_{20}N_2O]^{\cdot+}$, 40), 238 ($[M-C_{14}H_{22}N_2O_2]^{\cdot+}$, 61), 84 ($[C_5H_{10}N]^{\cdot+}$, 49), and 55 ($[C_3H_3O]^{\cdot+}$, 87). Anal. Found: C, 68.25; H, 6.50;

N, 11.34%. $C_{28}H_{32}N_4O_4 \cdot 0.25H_2O$ requires C, 68.20; H, 6.64; N, 11.36%.

Example 5

1,4-Bis[3-(4-morpholino)propionamido]anthracene-9,10-dione (BSU-1076)

Morpholine (2.1 g, 0.024 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate B (1 g, 2.4 mmol) in EtOH (60 mL). After 3 h of reflux, TLC (1:1 v/v EtOH-CH_2Cl_2) indicated reaction completion. The mixture was chilled to 0-5 °C and water (100 mL) was added; the precipitated solid was filtered, washed with water and dried in vacuo at 40 °C to give the title 1,4-bis(amido)anthraquinone (1.15 g, 93%) as a red-brown solid, mp 186-187 °C. The dihydrochloride salt was prepared in the form of a powder using the general procedure described for BSU-1062 above: mp 274-275 °C dec.; NMR δ 2.45 (t, J = 4.6 Hz, 8H, $N(CH_2CH_2)_2O$), 2.52-2.68 (ABq, 8H, $COCH_2CH_2N$), 3.61 (t, J = 4.6 Hz, 8H, $N(CH_2CH_2)_2O$), 7.95 (m, 2H, H-6,7), 8.20 (m, 2H, H-5,8), 8.88 (s, 2H, H-2,3), and 12.20 (s, 2H, D_2O removes, CONH); IR (KBr) 3415 (NH), 3157, 2957, 1697 (C=O), 1636 (quinone C=O), 1592, and 1584 cm^{-1}; UV/vis [CH_3OH, λ (log ϵ)], 254 (5.85), 314 (5.11), and 465 (4.89) nm; MS (rel intensity) m/z 520 ($[M]^{\cdot+}$, 20), 433 ($[M-C_4H_9NO]^{\cdot+}$, 15), 346 ($[M-C_8H_{18}N_2O_2]^{\cdot+}$, 17), 292 ($[M-C_{11}H_{20}N_2O_2]^{\cdot+}$, 37), 238 ($[M-C_{14}H_{22}N_2O_4]^{\cdot+}$, 35), 100 ($[C_5H_{10}NO]^{\cdot+}$, 100), 86 ($[C_4H_8NO]^{\cdot+}$, 55), and 56 ($[C_3H_4O]^{\cdot+}$, 46). Anal. Found: C, 64.63; H, 6.13; N, 10.72%. $C_{28}H_{32}N_4O_6$ requires C, 64.60; H, 6.20; N, 10.76%.

Example 6

1,4-Bis(4-piperidinobutyramido)anthracene-9,10-dione (BSU-9027)

1,4-Bis(4-chlorobutyramido)anthracene-9,10-dione

(BSU-9025)

To a stirred suspension of 1,4-diaminoanthraquinone (3.0 g, 12.6 mmol) and pyridine (0.5 ml) in toluene (500 ml) at 70 °C was added dropwise 4-chlorobutyryl chloride (6.7 ml, 60 mmol) in toluene (100 ml) over 1 hour. The mixture was stirred at 70 °C for 6 hours and filtered whilst still warm. The solids were washed with DCM (50 ml) and the combined filtrate evaporated *in vacuo*. The residue was dissolved in chloroform and treated with decolourising charcoal. Filtration and evaporation under reduced pressure afforded the crude product. Recrystallisation from DMF-EtOH (2:1 v/v) gave chloroamide **BSU-9025** (4.38 g, 78%) as dark red crystals; mp 210 °C; NMR δ($CDCl_3$) 2.27 (4H, quintet, J = 6.7, $CH_2CH_2CH_2$), 2.77 (4H, t, J = 6.7, $COCH_2$), 3.71 (4H, t, J = 6.7, CH_2Cl), 7.84 (2H, dd, J = 5.8, 3.3, H-6,7), 8.29 (2H, dd, J = 5.8, 3.3, H-5,8), 9.16 (2H, s, H-2,3), 12.63 (2H, s, NH); MS (rel intensity) *m/z* 447 (37), 343 (42), 239 (100); Calcd ($[M+1]^+$) 447.0878. Found 447.0870; Anal. Calcd ($C_{22}H_{20}N_2O_4Cl_2$): C, 59.07; H, 4.51; N, 6.26; Cl, 15.85. Found C, 59.09; H, 4.65; N, 6.24; Cl, 16.05.

1,4-Bis(4-piperidinobutyramido)anthracene-9,10-dione (BSU-9027)

To a stirred solution of 1,4-bis(4-chlorobutyramido)anthracene-9,10-dione **BSU-9025** (100 mg, 0.24 mmol) and NaI (0.1 g) in DMA (4 ml) at 70 °C was added piperidine (0.1 ml, 1 mmol). The mixture was stirred at 70 °C for 5 hours. The solvent was removed under reduced pressure and the residue dissolved in chloroform (25 ml), washed with water (10 ml) and dried. Evaporation gave the crude product which was recrystallised from EtOH to afford amide **BSU-9027** (50 mg, 38%) as red needles; mp 136-137 °C; NMR δ($CDCl_3$) 1.44 (4H, m, $(CH_2CH_2)_2CH_2$), 1.58 (8H, m, $N(CH_2CH_2)_2$), 2.00 (4H, quintet, J = 7.3, $COCH_2CH_2$),

2.41-2.61 (16H, m, $COCH_2CH_2CH_2N$ and $N(CH_2CH_2)_2$), 7.82 (2H, dd, J = 5.8, 3.3 H-6,7), 8.28 (2H, dd, J = 5.8, 3.3, H-5,8), 9.18 (2H, s, H-2,3), 12.54 (2H, s, NH); MS (rel intensity) m/z 545 (100), 459 (7), 307 (12); Calcd ([M+1]$^+$) 545.3128. Found 545.3120; Anal. Calcd ($C_{32}H_{40}N_4O_4$): C, 70.56; H, 7.40; N, 10.28. Found C, 70.26; H, 7.30; N, 10.23.

Preparative Method for anthraquinone free bases of Formula (II) and acid addition salts thereof:

Example 7

2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione (BSU-1060)

(A) *Aminolysis of 2,6-bis(3-chloropropionamido)anthraquinone*

Dimethylamine (20 mL, 0.112 mol as a 33% EtOH solution) was added dropwise during 15 min to a stirred, refluxing suspension of 2,6-bis(3-chloropropionamido)anthracene-9,10-dione (intermediate **A**; 4 g, 9.5 mmol; prepared by the published procedure of Agbandje et al., *J. Med. Chem.* 1992, *35*, 1418-1429) in EtOH (100 mL). After reflux for 4 h, thin-layer chromatography (TLC - silica gel; EtOH) indicated reaction completion and the mixture was chilled to 0-5 °C using an external ice-water bath. It was found that the reflux time is reduced to 2 h if a catalytic quantity of either sodium or potassium iodide (e.g. 0.5-2 g) is added to the initial reaction mixture. The solid that separated was removed by filtration and washed with dry ether to give the title compound (3.84 g, 92%) as an amorphous brown solid, mp >300 °C.

(B) *Preparation of acetic acid addition salt (General Procedure)*

A solution of the anthraquinone free base (1-2 mmol) in glacial acetic acid (15 mL) is heated to 50-60 °C, then

treated with decolourizing charcoal (250 mg) and filtered. Trituration of the clear filtrate with anhydrous ether gives a hygroscopic precipitate. This solid is digested repeatedly with dry ether (3 x 50 mL), filtered, washed with ether, and finally dried in vacuo (P_2O_5, <1 mm Hg) at 25 °C.

Treatment of the 2,6-bis(amido)anthraquinone using this general procedure gave the acetic acid addition salt (85% yield): mp >300 °C; NMR δ 2.19 (s, 12H, NCH_3), 2.51 (t, J = 5.2 Hz, 4H, $COCH_2CH_2N$), 2.58 (t, J = 5.2 Hz, 4H, $COCH_2CH_2N$), 8.04 (dd, J = 8.6 Hz, J = 1.9 Hz, 2H, H-3,7), 8.14 (d, J = 8.6 Hz, 2H, H-4,8), 8.41 (d, J = 1.9 Hz, 2H, H-1,5), and 10.67 (s, D_2O removes, 2H, CONH); IR (KBr) 3343 (NH), 2979, 2944, 2822, 2767, 1704 (C=O), 1659 (quinone C=O), 1576, and 1527 cm^{-1}; UV/vis [CH_3OH, l (log ϵ)], 233 (5.63), 277 (5.83), 305 (5.65), and 352 (5.18) nm; MS (rel intensity) m/z 437 ($[M+1]^{\cdot+}$, 2), 436 ($[M]^{\cdot+}$, 4), 391 ($[M-C_2H_7N]^{\cdot+}$, 13), 346 ($[M-C_4H_{14}N_2]^{\cdot+}$, 75), 292 ($[M-C_7H_{16}N_2O]^{\cdot+}$, 45), 238 ($[M-C_{10}H_{18}N_2O_2]^{\cdot+}$, 80), 56 ($[C_3H_4O]^{\cdot+}$, 100), and 45 ($[C_2H_7N]^{\cdot+}$, 100). Anal. Found: C, 65.30; H, 6.23; N, 12.29%. $C_{24}H_{28}N_4O_4 \cdot 0.25H_2O$ requires C, 65.36; H, 6.51; N, 12.70%.

Example 8

2,6-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione (BSU-1082)

Dipropylamine (7.3 g, 0.072 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **A** (3 g, 7.2 mmol) in EtOH (100 mL). TLC examination (silica gel; EtOH) after 9 h of reflux indicated quantitative removal of starting material. The mixture was chilled to 0-5 °C, and the solid precipitate was collected by filtration then washed with dry ether to give the title 2,6-bis(amido)anthraquinone (3.27 g, 84%) as an amorphous brown solid, mp 204-205 °C dec. Treatment

with acetic acid using the general procedure outlined above for BSU-1060 gave the diacetate salt: mp >200 °C dec. Anal. Found: C, 69.82; H, 8.16; N, 10.14%. $C_{32}H_{44}N_4O_4$ requires C, 70.04; H, 8.08; N, 10.21%.

Example 9

2,6-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione (BSU-1085)

Dibutylamine (9.3 g, 0.072 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **A** (3 g, 7.2 mmol) in EtOH (100 mL). After 9 h reflux, at which time TLC (silica gel; EtOH) indicated completion of reaction, the mixture was chilled to 0-5 °C. The solid that separated was removed by filtration, and washed with EtOH and dry ether to give the title compound (3.06 g, 71%) as an amorphous brown solid, mp 173-174 °C. The diacetate salt was prepared, using the general procedure outlined above for BSU-1060, in the form of a powder: mp 144-145 °C dec. Anal. Found: C, 71.35; H, 8.71; N, 9.21%. $C_{36}H_{52}N_4O_4$ requires C, 71.49; H, 8.67; N, 9.26%.

Example 10

2,6-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione (BSU-1078)

Pyrrolidine (5.1 g, 0.072 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **A** (3.0 g, 7.2 mmol) in EtOH (100 mL). After 4 h of reflux, at which time TLC (silica gel; EtOH) indicated completion of reaction, the mixture was chilled to 0-5 °C. The solid that separated was removed by filtration and washed with ether. Recrystallization from DMF-EtOH (4:1 v/v) afforded the title compound (3.3 g, 94%) as an amorphous brown solid, mp >300 °C dec. The diacetate salt (acetic acid addition salt) was prepared using the general procedure described for BSU-1060, as a

powder: mp >300 °C dec.; NMR δ 1.68 (m, 8H, pyrr. H-3,4), 2.50 (m, 8H, pyrr. H-2,5), 2.57 (t, *J* = 6.8 Hz, 4H, $COCH_2CH_2N$), 2.74 (t, *J* = 6.8 Hz, 4H, $COCH_2CH_2N$), 8.05 (dd, *J* = 8.6 Hz, *J* = 2.0 Hz, 2H, H-3,7), 8.15 (d, *J* = 8.6 Hz, 2H, H-4,8), 8.44 (d, *J* = 2.0 Hz, 2H, H-1,5), and 10.70 (s, D_2O removes, 2H, CONH); IR (KBr) 3348 (NH), 2963, 2790, 1704 (C=O), 1660 (quinone C=O), 1578, and 1523 cm^{-1}; UV/vis [CH_3OH, λ (log ε)], 233 (5.16), 277 (5.32), 305 (5.15), and 351 (4.65) nm; MS (rel intensity) *m/z* 488 ($[M]^{\cdot+}$, 4), 417 ($[M-C_4H_9N]^{\cdot+}$, 8), 346 ($[M-C_8H_{18}N_2]^{\cdot+}$, 34), 292 ($[M-C_{11}H_{20}N_2O]^{\cdot+}$, 20), 238 ($[M-C_{14}H_{22}N_2O_2]^{\cdot+}$, 7), 84 ($[C_5H_{10}N]^{\cdot+}$, 42), and 55 ($[C_3H_3O]^{\cdot+}$, 100). Anal. Found: C, 68.19; H, 6.57; N, 11.35%. $C_{28}H_{32}N_4O_4 \cdot 0.25H_2O$ requires C, 68.20; H, 6.64; N, 11.36%.

Example 11

2,6-Bis[2-(bis(2-hydroxyethyl)amino)acetamido]anthracene-9,10-dione (BSU-1065)

Diethanolamine (2.6 g, 0.025 mol) in DMF (5 mL) was added during 30 min to a stirred solution of 2,6-bis(2-chloroacetamido)anthracene-9,10-dione (1.00 g, 2.5 mmol; prepared by the published procedure of Agbandje et al., *J. Med. Chem.* 1992, *35*, 1418-1429) in DMF (40 mL) heated at 80 °C. After 4.5 h, TLC [silica gel; EtOH-$CHCl_3$ (3:7 v/v)] indicated completion of reaction. Chilling and trituration with ether gave a solid that was filtered, and washed with water then cold ethanol. The title compound (1.1 g, 80%) was obtained as a brown solid, mp 197-198 °C; NMR d 2.70 (t, *J* = 5.1 Hz, 8H, NCH_2CH_2OH), 3.41 (s, 4H, $COCH_2N$), 3.52 (br. q, *J* ~ 5.0 Hz, 8H, NCH_2CH_2OH), 4.81 (t, *J* = 4.9 Hz, D_2O removes, 4H, NCH_2CH_2OH), 8.08-8.20 (m, 4H, H-3,4,7,8), 8.48 (s, 2H, H-1,5), and 10.58 (s, D_2O removes, 2H, CONH); IR (KBr) ~3300 (br, OH), 3295 (br, NH), 2821, 1700 (C=O), 1673 (quinone C=O), 1589, and 1531 cm^{-1}; UV/vis [CH_3OH, λ (log ε)], 232 (5.41), 277 (5.59), 305 (5.55), and 349 (5.09) nm. Anal. Found: C, 57.62; H, 6.03; N, 10.40%.

$C_{26}H_{32}N_4O_8 \cdot 0.75H_2O$ requires C, 57.61; H, 6.23; N, 10.34%.

Example 12

2,6-Bis[3-[(2-hydroxyethyl)ethylamino]propionamido] anthracene-9,10-dione (BSU-6001)

2,6-Bis(3-chloropropionamido)anthracene-9,10-dione (intermediate **A**; prepared by the published procedure of Agbandje et al., J.Med. Chem., 1992, 35, 1418-1429) was treated with ethylaminoethanol according to the general aminolysis procedure described in Example 7 to give amide BSU 6001 (1.56g, 88%) as a pale yellow solid; NMR ($CDCl_3$) δ 0.97 (6H, t, J = 7.1Hz, CH_3), 2.55 (12H, m, NCH_2), 2.80 (4H, t, J = 6.5Hz, $COCH_2$), 3.46 (6H, t, J = 5.3Hz, CH_2OH), 8.03 (2H, dd, J = 8.6Hz, 2.1Hz, H-3,7), 8.14 (2H, d, J = 8.5Hz, H-4,8), 8.43 (2H, d, J = 2.1Hz, H-1,5), 10.78 (2H, s, NH), mp 183-184°C

Example 13

2,6-Bis[3-(hexamethyleneimino)propionamido]anthracene-9,10-dione (BSU-9080)

2,6-Bis(3-chloropropionamido) anthracene-9,10-dione (intermediate **A**; prepared by the published procedure of Agbandje et al., J.Med. Chem., 1992, 35, 1418-1429) was treated with hexamethyleneimine according to the general aminolysis procedure described in Example 7 to give amide BSU 9080 (1.28g, 65%) as a pale yellow solid; NMR ($CDCl_3$) δ 0.97 (6H, t, J = 7.1Hz, CH_3), 2.55 (12H, m, NCH_2), 2.80 (4H, t, J = 6.5Hz, $COCH_2$), 3.46 (6H, t, J = 5.38Hz, CH_2OH), 8.03 (2H, dd, J = 8.6Hz, 2.1Hz, H-3,7), 8.14 (2H, d, J = 8.5Hz, H-4,8), 8.43 (2H, d, J = 2.1Hz, H-1,5), 10.78 (2H, s, NH), mp >360°C

Example 14

2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione (BSU-9081)

2,6-Bis(3-chloropropionamido) anthracene-9,10-dione (intermediate **A**; prepared by the published procedure of Agbandje et al., J.Med. Chem., 1992, 35, 1418-1429) was treated with heptamethyleneimine according to the general aminolysis procedure described in Example 7 to give amide BSU 9081 (0.3g, 14%) as a yellow solid; NMR (DMSO) δ1.49(20H,m, CH_2), 2.51 (12H, m, NCH_2), 2.81 (t, J = 6.5Hz, 4H, $COCH_2$), 8.10 (2H, d, J = 8.7Hz, H-3,7), 8.16 (2H, d, J = 8.6Hz, H-4,8), 8.42 (2H, s, H-1,5), 10.56 (2H, s, NH), mp 258-259°C

Example 15

2,6-Bis[3-(*N,N*-diethyl-*N'*-methylethylenediamino)propionamido] anthracene-9,10-dione (BSU-9082)

2,6-Bis(3-chloropropionamido) anthracene-9,10-dione (intermediate **A**; prepared by the published procedure of Agbandje et al., J.Med. Chem., 1992, 35, 1418-1429) was treated with *N,N*-diethyl-*N'*-methylenediamine according to the general aminolysis procedure described in Example 7 to give amide BSU 9082 (0.3g, 26%) as a beige solid; NMR ($CDCl_3$) δ1.17(12H,t, J = 7.1Hz, CH_3), 2.31 (6H, s, NCH_3), 2.67 (2H, m, NCH_2), 3.14 (12H, m,CH_2), 8.06 (2H, d, J = 8.5Hz, H-3,7), 8.19 (2H, J = 8.5Hz, H-4,8), 8.48 (2H, s, H-1,5), 10.69 (2H, s, NH), mp 200-201°C

Example 16

2,6-Bis[3-(methylamino)propionamido]anthracene-9,10-dione (BSU-9083)

2,6-Bis(3-chloropropionamido) anthracene-9,10-dione (intermediate **A**; prepared by the published procedure of Agbandje et al., J.Med. Chem., 1992, 35, 1418-1429) was treated with methylamine according to the general aminolysis procedure described in Example 7 to give amide BSU 9083 (0.44g, 60%) as a pale brown solid; NMR (DMSO)

δ2.47(6H,s, CH_3), 2.70 (4H, t, J = 6.4Hz, $COCH_2$), 3.03 (4H, t, J = 6.3Hz, NCH_2), 8.03 (2H, d, J = 8.5Hz, H-3,7), 8.15 (2H, d, J = 8.4Hz, H-4,8), 8.45 (2H, s, H-1,5) 10.53 (2H, s, NH), mp 251-253°C.

Example 17

2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione (BSU-9084)

2,6-Bis(3-chloropropionamido) anthracene-9,10-dione (intermediate A; prepared by the published procedure of Agbandje et al., J.Med. Chem., 1992, 35, 1418-1429) was treated with azetidine according to the general aminolysis procedure described in Example 7 to give amide BSU 9084 (0.22g, 55%) as a brown solid; NMR (DMSO) δ2.34(4H,m, $CH_2CH_2CH_2$),2.77(4H,t, J = 6.7Hz, $COCH_2$), 3.48 (4H, t, J = 6.5Hz, NCH_2), 4.10 (8H, m, NCH_2), 8.03 (2H, dd, J = 8.7Hz, 1.6, H-3,7), 8.18 (2H, d, J = 8.5Hz, H-4,8), 8.48 (2H, d, J = 1.5Hz, H-1,5) 10.82 (2H, s, NH), mp 223-225°C.

Example 18

2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano)propionamido] anthracene-9,10-dione (BSU-9085)

2,6-Bis(3-chloropropionamido) anthracene-9,10-dione (intermediate A; prepared by the published procedure of Agbandje et al., J.Med. Chem., 1992, 35, 1418-1429) was treated with 1,3,3-trimethyl-6-azobicyclo[3.2.1]octane according to the general aminolysis procedure described in Example 7 to give amide BSU 9081 (0.3g, 14%) as a pale brown solid; NMR ($CDCl_3$) δ 1.00 (6H, s, CH_3), 1.11 (6H, s, CH_3), 1.25 (4H, m, CH_2) 1.34 (6H, s, CH_2), 1.46 (4H, m, CH_2), 1.72 (4H, m, CH_2), 2.22 (2H, m, CH), 2.50 (4H, t, J = 5.3Hz, NCH_2), 2.88 (4H, m, $COCH_2$), 3.29 (4H, m, CH_2), 8.09 (2H, d, J = 2.1Hz, H-1-5), 8.25 (2H, d, J = 8.5Hz, H-4,8), 8.35 (2H, d, J = 2.1Hz, 8.5Hz, H-3,7), 11.24 (2H, s, NH),

mp 255-256°C.

Example 19

2,6-Bis[3-(*N,N*-dimethylethylenediamino)propionamido] anthracene-9,10-dione (BSU-6004)

2,6-Bis(3-chloropropionamido) anthracene-9,10-dione (intermediate **A**; prepared by the published procedure of Agbandje et al., J.Med. Chem., 1992, 35, 1418-1429) was treated with *N,N*-dimethylethylenediamine according to the general aminolysis procedure described in Example 7 to give amide BSU-6004 (0.97g, 52%) as an orange solid; NMR ($CDCl_3$) δ 2.31 (12H, s, CH_3), 2.54 (8H, m,CH_2), 2.82 (4H, t, J = 6.1Hz, CH_2), 3.04 (4H, t, J = 5.3Hz, CH_2), 8.04 (2H, d, J = 2.0Hz, H-1,5), 8.24 (2H, d, J = 8.5Hz, H-4,8), 8.39 (2H, dd, J = 2.1Hz, 8.8Hz, H-3,7), 11.73 (2H, s, NH), mp >330°C.

Preparative method for quaternary ammonium salts of anthraquinones of formula (I):

Example 20

1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione *N,N'*-diethiodide (BSU-1068)

A mixture containing **BSU-1062** (500 mg, 1.15 mmol), iodoethane (5 mL, 0.05 mol) and acetone (20 mL) was stirred at 25 °C for 2 h. TLC (silica gel; EtOH) indicated completion of reaction. The solid which separates was filtered, washed with dry ether (3 x 20 mL), and dried in vacuo at 25 °C. The bis(ethylammonium) quaternary ethiodide salt (710 mg, 83%) was obtained as an amorphous orange powder, mp 199-200 °C; NMR δ 1.29 (t, J = 7.1 Hz, 6H, NCH_2CH_3), 3.08 (s, 12H, NCH_3), 3.21 (q, J = 7.1 Hz, 4H, NCH_2CH_3), 3.44 (t, J = 7.5 Hz, 4H, $COCH_2CH_2N$), 3.65 (t, J = 7.5 Hz, 4H, $COCH_2CH_2N$), 7.99 (m, 2H, H-6,7), 8.22 (m, 2H, H-5,8), 8.88 (s, 2H, H-2,3), and 12.20 (s, D_2O removes, 2H,

CONH); IR (KBr) 3436 (br, NH), 3011, 2687, 1702 (C=O), 1637 (quinone C=O), 1591 and 1500 cm^{-1}; UV/vis [CH_3OH, λ (log ε)], 217 (5.81), 257 (6.06), 313 (5.15), and 460 (4.86) nm. Anal. Found: C, 44.97; H, 4.92; N, 7.42; I, 33.78%. $C_{28}H_{38}N_4O_4I_2$ requires C, 44.93; H, 5.12; N, 7.49; I, 33.91%.

Example 21

1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1063)

A mixture containing **BSU-1062** (0.5 g, 1.15 mmol), iodomethane (5 mL, 0.08 mol) and acetone (20 mL) was stirred at 25 °C for 20 min. After this time, TLC indicated quantitative removal of the quinone reagent and completion of the reaction. The separated solid was filtered, washed with dry ether (3 x 20 mL) and finally dried in vacuo at 25 °C. The bis(methylammonium) quaternary methiodide salt (0.75 g, 92%) was obtained as an amorphous powder, mp 231-232 °C; NMR δ 3.15 (s, 18H, NCH_3), 3.23 (t, *J* = 7.6 Hz, 4H, $COCH_2CH_2N$), 3.70 (t, *J* = 7.6 Hz, 4H, $COCH_2CH_2N$), 7.99 (m, 2H, H-6,7), 8.22 (m, 2H, H-5,8), 8.87 (s, 2H, H-2,3), and 12.20 (s, D_2O removes, 2H, CONH); IR (KBr) 3436 (br, NH), 3011, 1698 (C=O), 1645 (quinone C=O), 1592 and 1505 cm^{-1}; UV/vis [CH_3OH, λ (log ε)], 221 (5.66), 256 (5.85), 318 (5.09), and 462 (4.85) nm; MS (FAB, rel intensity) *m/z* 593 ($[M+I]^{\cdot+}$, 13), 466 ($[M]^{\cdot+}$, 26), 451 ($[M-CH_3]^{\cdot+}$, 15), 436 ($[M-C_2H_6]^{\cdot+}$, 6), 407 ($[M-C_3H_9N]^{\cdot+}$, 58), 352 ($[M-C_6H_{12}NO]^{\cdot+}$, 19), and 238 ($[M-C_{12}H_{24}N_2O_2]^{\cdot+}$, 16). Anal. Found: C, 43.19; H, 4.69; N, 7.59; I, 35.11%. $C_{26}H_{34}N_4O_4I_2$ requires C, 43.35; H, 4.76; N, 7.78; I, 35.23%.

Example 22

1,4-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide (BSU-1080)

Treatment of the free base **BSU-1079** with iodomethane,

using the general procedure desribed for BSU-1061, gave the bisquaternary dimethiodide salt as an amorphous powder, mp 153-154 °C. Anal. Found: C, 48.28; H, 5.91; N, 6.55; I, 30.14%. $C_{34}H_{50}N_4O_4I_2 \cdot 0.5H_2O$ requires C, 48.52; H, 6.11; N, 6.66; I, 30.16%.

Example 23

1,4-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1087)

Treatment of the 1,4-bis(amido)anthraquinone free base **BSU-1084** with iodomethane, using the general procedure described for BSU-1061, gave the corresponding dimethiodide salt as an amorphous powder, mp 85-86 °C. Anal. Found: C, 50.52; H, 6.55; N, 6.19; I, 27.66%. $C_{38}H_{58}N_4O_4I_2 \cdot H_2O$ requires C, 50.34; H, 6.67; N, 6.18; I, 27.99%.

Example 24

1,4-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1075)

The bisquaternary dimethiodide salt was prepared in the form of an amorphous powder, mp 215-216 °C, by treatment of the free base 1,4-bis(amido)anthraquinone **BSU-1074** with iodomethane, using the general procedure outlined above for BSU-1061. Anal. Found: C, 46.52; H, 4.93; N, 7.11; I, 32.99%. $C_{30}H_{38}N_4O_4I_2$ requires C, 46.65; H, 4.96; N, 7.25; I, 32.86%.

Example 25

1,4-Bis[3-(4-morpholino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1077)

Treatment of the bis(morpholine)-substituted derivative **BSU-1076** with iodomethane, using the general procedure outlined above for BSU-1061, gave the dimethiodide salt as an amorphous powder, mp 234-235 °C. Anal. Found: C, 44.58;

H, 4.72; N, 6.84; I, 31.54%. $C_{30}H_{38}N_4O_6I_2$ requires C, 44.79; H, 4.76; N, 6.96; I, 31.55%.

Example 26

1,4-Bis(4-piperidinobutyramido)anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9031)

A mixture of amino amide BSU-9027 (56 mg, 0.1 mmol) and iodomethane (0.33 ml, 5 mmol) in DCM (10 ml) was stirred at room temperature for 24 h. The resulting mixture was filtered, washed with dry ether and dried *in vacuo* to give dimethiodide **BSU-9031** (78 mg, 97.5%) as a red solid; mp 272 °C dec. Anal. Calcd ($C_{34}H_{46}N_4O_4I_2 \cdot 1.5H_2O$): C, 47.73; H, 5.77; N, 6.55; I, 29.66. Found C, 47.84; H, 5.63; N, 6.45; I, 29.76.

Preparative Method for quaternary ammonium salts of anthraquinones of Formula (II):

Example 27

2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1061)

General Quaternisation Procedure

A mixture containing **BSU-1060** (1 g, 2.56 mmol), iodomethane (10 mL, 0.16 mol) and acetone (40 mL) is stirred at 25 °C for 2 h. After this time TLC (silica gel; EtOH) indicated completion of reaction. The solid which separates was filtered, washed with dry ether (3 x 60 mL), and dried in vacuo at 25 °C. The bis(methylammonium) quaternary methiodide salt (1.63 g, 99%) is recovered as an amorphous powder, mp 266-267 °C dec.; NMR δ 3.01 (t, J = 7.3 Hz, 4H, $COCH_2CH_2N$), 3.12 (s, 18H, NCH_3), 3.70 (t, J = 7.3 Hz, 4H, $COCH_2CH_2N$), 8.04 (dd, J = 8.5 Hz, J = 1.4 Hz, 2H, H-3,7), 8.19 (d, J = 8.5 Hz, 2H, H-4,8), 8.47 (d, J = 1.4 Hz, 2H, H-1,5), and 10.87 (s, D_2O removes, 2H, CONH); IR (KBr) 3440 (br, NH), 3098, 3050, 1700 (C=O), 1666

(quinone C=O), 1590, and 1533 cm^{-1}; UV/vis [CH_3OH, λ (log ε)], 220 (5.82), 277 (5.84), 303 (5.67), and 349 (5.14) nm. Anal. Found: C, 43.81; H, 4.77; N, 7.61; I, 35.21%. $C_{26}H_{34}N_4O_4I_2$ requires C, 43.35; H, 4.76; N, 7.78; I, 35.23%.

Example 28

2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione *N,N'*-diethiodide (BSU-1067)

A mixture containing **BSU-1060** (200 mg, 0.512 mmol), iodoethane (2 mL, 0.02 mol) and acetone (10 mL) was stirred at 25 °C for 24 h. After this time TLC (silica gel; EtOH) indicated reaction completion. The solid which separates was filtered, washed with dry ether (3 x 10 mL), and dried in vacuo at 25 °C. The bis(ethylammonium) quaternary iodide salt (310 mg, 91%) was recovered as an amorphous powder, mp 226-227 °C dec. Anal. Found: C, 44.08; H, 5.05; N, 7.12; I, 33.52%. $C_{28}H_{38}N_4O_4I_2 \cdot 0.75H_2O$ requires C, 44.14; H, 5.23; N, 7.35; I, 33.31%.

Example 29

2,6-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1083)

Treatment of the free base compound **BSU-1082** with iodomethane, using the general procedure outlined above for BSU-1061, gave the corresponding bisquaternary dimethiodide salt in the form of an amorphous powder, mp 203-204 °C dec. Anal. Found: C, 48.80; H, 5.98; N, 6.62; I, 29.98%. $C_{34}H_{50}N_4O_4I_2$ requires C, 49.05; H, 6.05; N, 6.73; I, 30.48%.

Example 30

2,6-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1086)

Analogous treatment of the diamine free base **BSU-1085** with iodomethane, using the general procedure outlined

above for BSU-1061, gave the dimethiodide salt as an amorphous powder, mp 205-206 °C. Anal. Found: C, 51.31; H, 6.55; N, 6.23; I, 28.39%. $C_{38}H_{58}N_4O_4I_2$ requires C, 51.36; H, 6.58; N, 6.30; I, 28.56%.

Example 31

2,6-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1081)

Alkylation treatment of the pyrrolidine free base BSU-1078 with iodomethane, using the general procedure outlined above for BSU-1061, gave the corresponding dimethiodide salt as an amorphous powder, mp 285-286 °C dec. Anal. Found: C, 46.44; H, 4.94; N, 7.06; I, 32.60%. $C_{30}H_{38}N_4O_4I_2$ requires C, 46.65; H, 4.96; N, 7.25; I, 32.86%.

Example 32

2,6-Bis[3-[(2-hydroxyethyl)ethylamino]propionamido] anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-6002)

Treatment of the free base BSU 6001 with iodomethane using the general procedure described in Example 27 gave the corresponding bisquaternary dimethiodide salt BSU 6002 as a yellow solid (0.29, 94%); mp 183-184 °C.

Example 33

2,6-Bis[3-(hexamethyleneimino)propionamido]anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9087)

Treatment of the free base BSU 9080 with iodomethane using the general procedure described in Example 27 gave the corresponding bisquaternary dimethiodide salt BSU 9087 as a yellow solid (0.24, 75%); mp 237-238°C.

Example 34

2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9088)

Treatment of the free base BSU 9084 with iodomethane

using the general procedure described in Example 27 gave the corresponding bisquaternary dimethiodide salt BSU 9088 as a brown solid (0.02g, 31%); mp >350°C.

Example 35

2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9089)

Treatment of the free base BSU 9081 with iodomethane using the general procedure described in Example 27 gave the corresponding bisquaternary dimethiodide salt BSU 9089 as a yellow solid (0.09g, 86%); mp 190-191°C.

Example 36

2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano)propionamido] anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9091)

Treatment of the free base BSU 9085 with iodomethane using the general procedure described in Example 27 gave the corresponding bisquaternary dimethiodide salt BSU 9091 as a yellow solid (0.05, 10%); mp 248-249°C.

Example 37

2,6-Bis[3-(*N,N*-diethyl-*N'*-methylethylenediamino)propionamido] anthracene-9,10-dione *N,N'*-Dimethiodide (BSU-9097)

Treatment of the free base BSU 9082 with iodomethane using the general procedure described in Example 27 gave the corresponding bisquaternary dimethiodide salt BSU 9097 as a yellow solid (0.02g, 55%); mp 198-199°C.

Preparation of Maleate Salts

Example 38

2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione maleate salt (BSU-9086)

To a stirred solution of the free base BSU 9080 (2-4mmol) in acetone (50mL) was added maleic acid (1 equivalent) in methanol (5mL) and the mixture refluxed for 2hrs. The resultant mixture was then cooled to 0°C and the precipitated solid filtered, washed repeatedly with diethyl ether and dried in vacuo. The title compound was obtained as a yellow solid (0.28, 93%); mp 203-204°C.

Example 39

2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione maleate salt (BSU-9090)

Treatment of the free base BSU 9084 with maleic acid using the general procedure described in Example 38 gave the corresponding salt BSU 9090 as a brown solid (0.02g, 33%); mp 229-230°C.

Example 40

2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano)propionamido] anthracene-9,10-dione maleate salt (BSU-9092)

Treatment of the free base BSU 9085 with maleic acid using the general procedure described in Example 38 gave the corresponding salt BSU 9092 as a yellow solid (0.30g, 60%); mp 204-205°C.

Example 41

2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione maleate salt (BSU-9093)

Treatment of the free base BSU 9081 with maleic acid using the general procedure described in Example 38 gave the corresponding salt BSU 9093 as a yellow solid (0.96g, 98%); mp 191-192°C.

Example 42

2,6-Bis[3-(*N*,*N*-diethyl-*N'*-

methylethylenediamino)propionamido] anthracene-9,10-dione maleate salt (BSU-9094)

Treatment of the free base BSU 9082 with maleic acid using the general procedure described in Example 38 gave the corresponding salt BSU 9094 as a yellow solid (0.01g, 27%); mp 185-185°C.

Preparative method for anthraquinone free bases of formula (III) and addition salts thereof:

Example 43

1,4-Bis[3-(2-hydroxymethyl-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1057)

2-Piperidinemethanol (3.45 g, 0.03 mol) in EtOH (10mL) was added during 15 min to a stirred, refluxing suspension intermediate **B** (1.00 g, 2.56 mmol) in EtOH (60 mL). After 3 h reflux, at which time TLC (silica gel; EtOH-CH_2Cl_2 7:3 v/v as eluent) indicated completion of reaction, the mixture was concentrated to 30 mL and chilled to 0-5 °C. The solid that separated was removed by filtration and washed with dry ether to give the product (0.95 g, 70%) as an amorphous red-brown solid, mp 125-126 °C. Anal. Found: C, 64.32; H, 7.06; N, 9.47%. $C_{32}H_{40}N_4O_6 \cdot H_2O$ requires C, 64.63; H, 7.12; N, 9.42%.

Example 44

1,4-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione (BSU-1070)

Diethylamine (0.88 g, 0.012 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **B** (0.5 g, 1.2 mmol) in EtOH (40 mL). After 2.5 h reflux, at which time TLC [silica gel; EtOH-CH_2Cl_2 (1:1 v/v) as eluent] indicated completion of reaction, the mixture was chilled to 0-5 °C and water (50 mL) was added. The precipitate was collected by filtration, washed with

water and dried in vacuo at 40 °C. The bis(amido)anthraquinone (0.51 g, 88%) was obtained as a red-brown solid, mp 141-142 °C. Using the general procedure outlined for BSU-1062 above, the free base was converted to the corresponding dihydrochloride salt: mp 254-255 °C. Anal. Found: C, 67.74; H, 7.25; N, 11.28%. $C_{28}H_{36}N_4O_4 \cdot 0.25H_2O$ requires C, 67.65; H, 7.40; N, 11.27%.

Example 45

1,4-Bis[3-(2-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1064)

2-Piperidineethanol (3.88 g, 0.03 mol) in EtOH (10mL) was added during 15 min to a stirred, refluxing suspension of intermediate **B** (1 g, 2.56 mmol) in EtOH (60 mL). After 2 h of reflux, at which time TLC (EtOH-CH_2Cl_2 7:3 v/v as eluent) indicated completion of reaction, the solvent was removed and the residue treated with dry ether (20 mL). Filtration and recrystallization from EtOH afforded the title compound (1.06 g, 74%) as a brown solid, mp 121-122 °C. The corresponding dihydrochloride salt was prepared using the general procedure outlined for BSU-1062 above: mp 215-216 °C. Anal. Found: C, 64.13; H, 7.24; N, 8.76%. $C_{34}H_{46}N_4O_6 \cdot 1.5H_2O$ requires C, 64.43; H, 7.79; N, 8.84%.

Example 46

1,4-Bis[3-(1-piperidino)propionamido]anthracene-9,10-dione (BSU-1071)

Piperidine (2.0 g, 0.024 mol) in EtOH (10 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **B** (1 g, 2.4 mmol) in EtOH (60 mL). After 50 min of reflux, at which time TLC (EtOH-CH_2Cl_2 1:1 v/v as eluent) indicated completion of reaction, the mixture was chilled to 0-5 °C and water (100 mL) was added. The solid was collected by filtration, washed with water and dried in vacuo. The 1,4-bis(amido)anthraquinone (1.12 g, 91%)

was obtained as a red-brown solid, mp 165-166 °C. The dihydrochloride salt was prepared as a powder using the general procedure described above for BSU-1062: mp >270 °C dec. Anal. Found: C, 69.32; H, 6.94; N, 10.72%. $C_{30}H_{36}N_4O_4 \cdot 0.25H_2O$ requires C, 69.19; H, 7.06; N, 10.75%.

Preparative method for anthraquinone free bases of formula (IV) and acid addition salts thereof:

Example 47

2,6-Bis[3-(2-hydroxymethyl-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1040)

2-Piperidinemethanol (25 g, 0.22 mol) in EtOH (50 mL) was added during 15 min to a stirred, refluxing suspension of intermediate **A** (8 g, 0.019 mol) in EtOH (250 mL). After 22 h reflux, TLC (silica gel; EtOH) indicated completion of reaction and the mixture was chilled to 0-5 °C. The solid that separated was removed by filtration, washed with dry ether and diried in vacuo to give the title bis(amide) quinone compound (9.88 g, 90%) as an amorphous brown solid, mp 216-217 °C.

Example 48

2,6-Bis[3-(2-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1035)

2-Piperidineethanol (38 g, 0.29 mol) was added during 15 min to a stirred, refluxing suspension of intermediate **A** (10.0 g, 0.023 mol) in EtOH (300 mL). After 16 h of reflux, at which time TLC (silica gel; EtOH) indicated completion of reaction, the mixture was chilled to 0-5 °C. The solid that separated was removed by filtration and washed with dry ether to give the product (12.61 g, 87%) as an amorphous brown solid, mp 211-212 °C.

Example 49

2,6-Bis[3-(4-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione (BSU-1038)

4-Piperidineethanol (12.92 g, 0.1 mol) was added during 15 min to a stirred, refluxing suspension of intermediate **A** (5.0 g, 0.012 mol) in EtOH (150 mL). After 5 h reflux, at which time TLC (silica gel; EtOH) indicated completion of reaction, the mixture was chilled to 0-5 °C using an external ice-water bath. The solid that separated was removed by filtration and washed with dry ether to give the title compound (6.69 g, 93%) as an amorphous brown powder, mp 237-238 °C.

Example 50

2,6-Bis[3-(bis(2-hydroxyethyl)amino)propionamido] anthracene-9,10-dione (BSU-1041)

Diethanolamine (25 g, 0.24 mol) in EtOH (100 mL) was added during 30 min to a stirred, refluxing suspension of intermediate **A** (8 g, 0.019 mol) in EtOH (150 mL). After 20 h reflux, solvent removal gave a brown hygroscopic residue which was digested in 2-propanol (100 mL), triturated with ether (500 mL) and recovered by filtration. After two further such treatments, the solid was washed with ether (3 x 100 mL) and dried. Recrystallization from aqueous EtOH (10% v/v), with charcoal treatment, gave the title compound (9.50 g, 89%) as yellow prisms, mp 159-160 °C.

Preparative method for quaternary ammonium salts of anthraquinones of formula (III):

Example 51

1,4-Bis[3-(2-hydroxymethyl-1-piperidino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1058)

Treatment of **BSU-1057** with iodomethane using the general procedure outlined above for BSU-1061 gave the corresponding bisquaternary dimethiodide salt as an amorphous powder, mp 175-176 °C. Anal. Found: C, 46.89; H, 5.23; N, 6.49; I, 29.79%. $C_{34}H_{45}N_4O_6I_2 \cdot 0.25H_2O$ requires C, 47.21; H, 5.42; N, 6.48; I, 29.39%.

Example 52

1,4-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1073)

Using the general procedure outlined above for BSU-1061, the free base **BSU-1070** was converted to the bisquaternary dimethiodide salt in the form of an amorphous powder, mp 224-225 °C. Anal. Found: C, 46.34; H, 5.41; N, 7.10; I, 32.67%. $C_{30}H_{42}N_4O_4I_2$ requires C, 46.40; H, 5.45; N, 7.22; I, 32.69%.

Example 53

1,4-Bis[3-(1-piperidino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1072)

Treatment of free base **BSU-1071** with iodomethane, using the general procedure described above for BSU-1061, gave the corresponding bisquaternary dimethiodide salt as an amorphous powder, mp 205-206 °C. Anal. Found: C, 46.81; H, 5.24; N, 6.76; I, 30.64%. $C_{32}H_{42}N_4O_4I_2 \cdot H_2O$ requires C, 46.96; H, 5.42; N, 6.84; I, 31.01%.

Preparative Method for quaternary ammonium salts of anthraquinones of formula (IV):

Example 54

2,6-Bis[3-(2-(2-hydroxymethyl)-1-piperidino)propionamido] anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1051)

A mixture containing **BSU-1040** (1.5 g, 2.6 mmol), iodomethane (10 mL, 0.16 mol) and acetone (50 mL) was

stirred at 25 °C for 24 h. A further 3 h at reflux was required to effect completion of the reaction as judged by TLC (silica gel; EtOH). After cooling to 0-5 °C, the mixture is filtered and the solid washed with dry ether (3 x 60 mL), and dried in vacuo at 25 °C. The bis(methylammonium) quaternary methiodide salt (2.13 g, 95%) was recovered as an amorphous powder, mp >210 °C dec. Anal. Found: C, 47.38; H, 5.38; N, 6.45; I, 29.31%. $C_{34}H_{46}N_4O_6I_2$ requires C, 47.45; H, 5.39; N, 6.51; I, 29.49%.

Example 55

2,6-Bis[3-(4-(2-hydroxyethyl)-1-piperidino)propionamido] anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1050)

A mixture containing **BSU-1038** (1.5 g, 2.5 mmol), iodomethane (10 mL, 0.16 mol) and acetone (50 mL) is stirred at 25 °C for 24 h. A further 8 h at reflux was required for completion of reaction (TLC: silica gel; EtOH). After cooling to 0-5 °C, the mixture is filtered and the solid washed with dry ether (3 x 60 mL), and dried in vacuo at 25 °C. The bis(methylammonium) quaternary methiodide salt (1.91 g, 86%) was recovered as an amorphous powder, mp 183-184 °C. Anal. Found: C, 49.03; H, 5.62; N, 6.35; I, 28.72%. $C_{36}H_{50}N_4O_6I_2$ requires C, 48.66; H, 5.67; N, 6.31; I, 28.56%.

Example 56

2,6-Bis[3-(bis(2-hydroxyethyl)amino)propionamido] anthracene-9,10-dione *N,N'*-dimethiodide (BSU-1052)

A mixture containing **BSU-1041** (0.50 g, 0.9 mmol), iodomethane (5 mL, 0.08 mol) and acetone (15 mL) was stirred at 25 °C for 24 h. After this time, TLC (silica gel; EtOH) indicated reaction completion. The separated solid was collected by filtration, washed with dry ether (3 x 60 mL), and finally dried in vacuo at 25 °C. The bis(methylammonium) quaternary methiodide salt (0.71 g,

93%) was recovered as an amorphous powder, mp 167-168 °C. Anal. Found: C, 43.23; H, 5.01; N, 6.67; I, 30.60%. $C_{30}H_{42}N_4O_8I_2$ requires C, 42.87; H, 5.04; N, 6.67; I, 30.20%.

Section B - Biological Assay

An "in vitro" Telomeric repeat amplification protocol" TRAP assay using a standard telomerase protein extract from A2780 human ovarian carcinoma cells was performed. In previous experiments, A2780 and A2780cisR cells, where the latter represent a derived cisplatin-resistant strain, have been shown to exhibit telomerase activity.

"in vitro" TRAP assay.

A modified TRAP assay (Mieczyslaw *et al*, Methods in Cell Science, 17: 1-15, 1995) was used involving quantitative PCR and harvesting of radiolabelled telomeric TTAGGG repeats on filters and quantification by liquid scintillation counting.

A2780 cells were lysed in a CHAPS lysis buffer which comprises 0.5% CHAPS (3-[(3-cholamidopropyl)-dimethylammino]-1-propanesulfonate), 10mM Tris-HCl [pH 7.5], 1mM $MgCl_2$, 1mM EGTA, 5mM β mercaptoethanol, 10% glycerol, 0.1mM AEBSF [freshly added]). 0.04 μg of protein extract from A2780 cells in CHAPS lysis buffer was added to a PCR master mix in sterile Eppendorfs. The PCR master mix contains:

26.95μl sterile water (to give final volume of 34μl); 4μl TRAP buffer (final concentration: 20mM Tris-HCl (pH 8.3), 68mM KCl, 1.5mM $MgCl_2$, 1mM EDTA, 0.05% Tween 20); 1.25μl 2mM dNTP's; 1μl TS "forward" left primer (100μg/ml); 0.5μl BSA at 100μg/ml; and 3μCi α-^{32}P dCTP

(at 10mCi/ml = 0.3μl)
The forward primer was of the following sequence:
5'AATCCGTCGAGCAGAGTT 3'.

The following controls were run in each assay:
A. lysis buffer (2μl).
B. Heat inactivation control (85° for 10 mins).
C. 2μl of "half-strength" protein extract (4μl of 125μg/ml) = 0.2μg
D. untreated protein alone (0.04μg protein) (2μl)
E. 2μl of quarter strength protein extract to check for quantitation.

4μl of drug dissolved in water at 500μM (or water) was then added at final concentrations of 50, 20, 10, 5 and 1μM.

These samples were then transferred to a PCR machine and held at 25°C for 20mins followed by 80°C for 5 mins. (for the taq control drug was added at final concentration of 50μM at this stage). The following "hot-start" PCR mix was then added to each tube:

7.6μl water
1μl CX reverse primer (100μg/ml)
primer = 3' AATCCCAATCCCAATCCCAATCCC 5'
1μl 10X TRAP buffer
0.4μl of 5U/μl Taq polymerase

and samples subjected to 31 PCR cycles of 94°C denaturing 30s; 50°C annealing 30s; 72°C 1 min.
Samples were then quickly pulse vortexed and 40μl of PCR reaction transferred into a 1.5ml eppendorf tube. 800μl of 5% trichloroacetic acid (TCA) with 20mM tetrasodium pyrophosphate was added and samples left for 1hr on ice. TCA-precipitated PCR products were then harvested on

Whatman filters (Millipore Unit) and filters washed with 10ml 5% TCA mix and 10ml 70% ethanol for 5 mins to dryness. The amount of radioactivity present on each filter was then determined by liquid scintillation counting. Results for each agent were expressed relative to the untreated protein alone control (minus heat inactivation control).

Table 1 below shows the assay results obtained for a selection of the anthraquinones of the invention and a selection of known anthraquinones of fomulae (III) and (IV) and their salts.

Table 1

Anthraquinone of Example No.	BSU Number	Telomerase Inhibition (CONC)					Trap Assay 50% INHIB IC_{50} Values
		(50μM)	(20μM)	(10μM)	(5μM)	(1μM)	
*	BSU 1021	99.1	97.8	92	64.7	10.6	4.5
*	BSU 1022	9.2	3.7	0.2	0	0	>50
*	BSU 1024	38.3	25.6	15.9	6.9	0.2	>50
*	BSU 1028	25.3	16.4	9.5	8.3	0.5	>50
*	BSU 1043	88.7	68.1	17.5	11.5	0	16.5
50	BSU 1058	100	87.2	54	7.9	0	9.4
27	BSU 1061	86.4	56.2	34.2	2.1	0	17.3
52	BSU 1072	94.1	74.2	45.7	22.3	0	11.1
24	BSU 1075	100	88	62.2	50.1	41.5	5.0
5	BSU 1076	68.6	32.9	16.7	0	0	33.5
25	BSU 1077	56.8	34.2	6.7	0	0	34.5
2	BSU 1079	49.7	25.4	0	0	0	50
8	BSU 1082	35	30.4	15.5	7.7	0	>50

BSU 1021 is 2,6-Bis(3-(1-piperidino)propionamido) anthracene-9,10-dione diacetate;

BSU 1022 is 2,6-Bis(2-(4-morpholino)acetamido)anthracene-9,10-dione diacetate;

BSU 1024 is 2,6-Bis(2-diethylaminoacetamido)anthracene-9,10-dione diacetate;

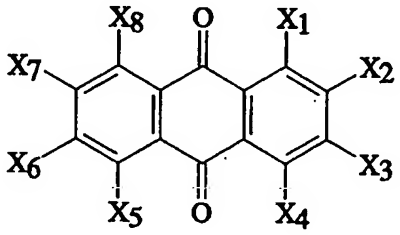
BSU 1028 is 2,6-Bis(3-(4-morpholino)propionamide) anthracene-9,10-dione diacetate; and

BSU 1043 is 2,6-Bis(3-(4-(2-hydroxyethyl)-1-piperazino)-propionamido)anthracene-9,10-dione diacetate.

The synthesis of these anthraquinone salts is described in WO91/00265. These anthraquinone salts are salts of compounds of formula (III) of the present invention.

CLAIMS

1. An anthraquinone of formula (I) or a pharmaceutically acceptable acid addition salt or quaternary ammonium salt thereof:



(I)

in which:

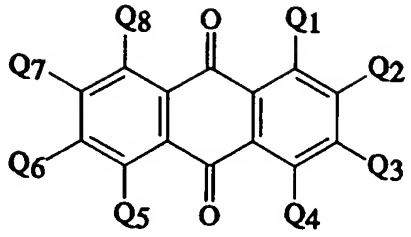
each of X_1 and X_4, which are the same or different, is $HNCO(CH_2)_nNR^1R^2$, wherein each of R^1 and R^2, which are the same or different, is an unsubstituted or substituted alkyl group or R^1 and R^2 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted heterocyclic group, and n is an integer of from 1 to 6;

each of X_2, X_3, X_5, X_6 X_7 and X_8, which are the same or different, is H, an unsubstituted or substituted alkyl group or halogen;

provided that:

when X_1 and X_4 are both $HNCO(CH_2)_nNR^1R^2$, and X_2, X_3, X_5, X_6, X_7 and X_8 are each H and n is 2, either R^1 and R^2 do not both represent ethyl, or R^1 and R^2 together with the nitrogen atom to which they are attached do not represent 1-piperidino or 2-hydroxymethyl-piperidino;

or an anthraquinone of formula (II) or a pharmaceutically acceptable acid addition salt or quaternary ammonium salt thereof:



in which:

each of Q_2 and Q_6, which are the same or different, is $HNCO(CH_2)_nNR^3R^4$, wherein each of R^3 and R^4, which are the same or different, is an unsubstituted or substituted alkyl group or R^3 and R^4 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted heterocyclic group, and n is an integer of from 1 to 6;

each of Q_1, Q_3, Q_4, Q_5, Q_7 and Q_8, which are the same or different is H, OH, an amino or substituted amino group, an unsubstituted or substituted alkyl group or halogen;

provided that:

when Q_2 and Q_6 are both $HNCO(CH_2)_nNR^3R^4$, and Q_1, Q_3, Q_4, Q_5, Q_7 and Q_8 are each H and n is 1, 2 or 3, either NR^3R^4 is not $N(CH_2)CH_3)_2$ or $N(CH_2CH_2OH)_2$ or R^3 and R^4 together with the nitrogen atom to which they are attached do not represent piperidino, morpholino, 4-methylpiperazino, 2-hydroxymethyl-piperidino , 2-hydroxyethyl-piperazino or 4-hydroxyethyl-piperidino.

2. A compound according to claim 1, wherein both groups R^1 are the same and both groups R^2 are the same or both groups R^3 are the same and both groups R^4 are the same.

3. A compound according to claim 1, wherein X_2, X_3, X_5, X_6, X_7 and X_8 or Q_1, Q_3, Q_4, Q_5, Q_7 and Q_8 are each H.

4 A compound according to any one of the preceding claims wherein n is 2.

5. A compound according to claim 1 wherein R^1 and R^2

or R^3 and R^4 are the same.

6. A compound according to any one of claim 1 wherein R^1 and R^2 or R^3 and R^4 together with the nitrogen atom to which they are attached represent a substituted or unsubstituted hexamethyleneimino, heptamethyleneimino, azetidino, pyrrolidino, morpholino or piperidino group.

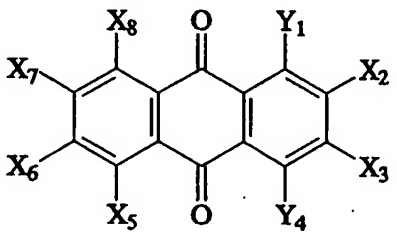
7. A compound according to claim 1 selected from:
1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione;
1,4-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione; 1,4-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione;
1,4-Bis[3-(pyrrolidino)propionamido]anthracene-9,10-dione;
1,4-Bis[3-(4-morpholino)propionamido]anthracene-9,10-dione;
1,4-Bis(4-piperidinobutyramido)anthracene-9,10-dione;
2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione;
2,6-Bis[2-(bis(2-hydroxyethyl)amino)acetamido]anthracene-9,10-dione;
2,6-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-[(2-hydroxyethyl)ethylamino]propionamido]anthracene-9,10-dione;
2,6-Bis[3-(hexamethyleneimino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(*N*,*N*-diethyl-*N'*-methylethylenediamino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(methylamino)propionamido]anthracene-9,10-dione;

2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano) propionamido] anthracene-9,10-dione;
2,6-Bis[3-(*N,N*-dimethylethylenediamino) propionamido]anthracene-9,10-dione;
1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide;
1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione N,N'-diethiodide;
1,4-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide;
1,4-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide;
1,4-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione N,N'-dimethiodide;
1,4-Bis[3-(4-morpholino)propionamido]anthracene-9,10-dione N,N'-dimethiodide;
1,4-Bis(4-piperidinobutyramido)anthracene-9,10-dione N,N^1-dimethiodide;
2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide;
2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione N,N'-diethiodide;
2,6-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide;
2,6-Bis[3-(dibutylamino)propionamido]anthracene-9,10-dione N,N'-dimethiodide;
2,6-Bis[3-(1-pyrrolidino)propionamido]anthracene-9,10-dione N,N'-dimethiodide.
2,6-Bis[3-[(2-hydroxyethyl)ethylamino]propionamido] anthracene-9,10-dione *N,N'*-dimethiodide;
2,6-Bis[3-(hexamethyleneimino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide;
2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide;

2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione *N,N'*-dimethiodide;
2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano) propionamido]anthracene-9,10-dione *N,N'*-dimethiodide;
2,6-Bis[3-(*N,N*-diethyl-*N'*-methylethylenediamino) propionamido] anthracene-9,10-dione *N,N'*-dimethiodide;
2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione maleate;
2,6-Bis[3-(azetidino)propionamido]anthracene-9,10-dione maleate;
2,6-Bis[3-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octano) propionamido] anthracene-9,10-dione maleate;
2,6-Bis[3-(heptamethyleneimino)propionamido]anthracene-9,10-dione maleate;
2,6-Bis[3-(*N,N*-diethyl-*N'*-methylethylenediamino) propionamido] anthracene-9,10-dione maleate.

8. A process for the production of an anthraquinone according to claim 1, which process comprises:

i) reacting a intermediate of formula (B):



(B)

in which:

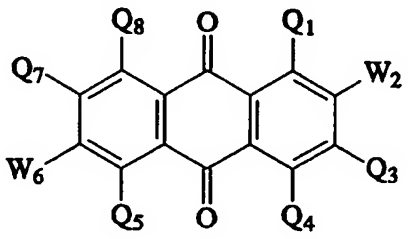
each of Y_1 and Y_4, which are the same or different, is $HNCO(CH_2)_nZ$, wherein Z is a leaving group and n is an integer of from 1 to 6, and X_2, X_3, X_5, X_6, X_7 and X_8 are as defined in claim 1;

with a compound of formula (C):

R^1R^2NH (C)

wherein R^1 and R^2 are as defined in claim 1; or

ii) reacting a intermediate of formula (A):



(A)

in which:

each of W_2 and W_6, which are the same or different, is $HCO(CH_2)_nZ$, wherein Z is a leaving group and n is an integer of from 1 to 6, and Q_1, Q_3, Q_4, Q_5, Q_7 and Q_8 are as defined in claim 1;

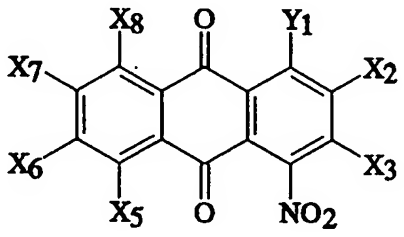
with a compound of formula (D):

$$R^3R^4NH \qquad (D)$$

wherein R^3 and R^4 as defined in claim 1.

9. A process for producing an anthraquinone of formula (I) as defined in claim 1 in which the two groups R^1 are not the same and/or the two groups R^2 are not the same, which process comprises:

(i) reacting an intermediate of formula (B'):



(B')

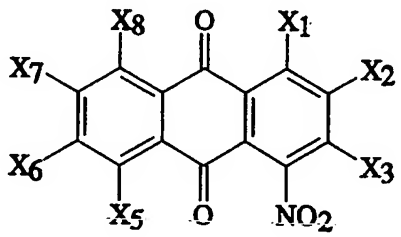
in which:

Y_1 is $HNCO(CH_2)_nZ$, wherein Z is a leaving group and n is an integer of from 1 to 6, and X_2, X_3, X_5, X_6, X_7 and X_8 are as defined in claim 1;

with a compound of formula (C):

$$R^1R^2NH \qquad (C)$$

wherein R^1 and R^2 are as defined in claim 1, to give a compound of formula (E):

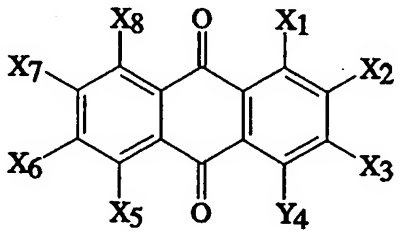


(E)

wherein X_1 is as defined in claim 1;

(ii) converting the NO_2 group to an NH_2 group;

(iii) reacting the product of step (ii) with $Z(CH_2)_nCOZ$ wherein Z is a leaving group and n is an integer of from 1 to 6, to give a product of formula (F):



in which Y_4 is $HNCO(CH_2)_nZ$;

(iv) reacting the product of step (iii) with a compound of formula (C'):

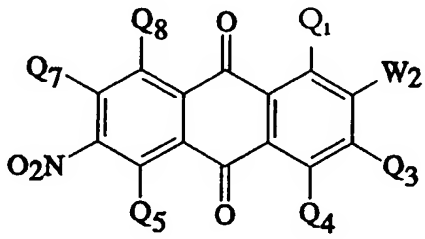
$$R^1R^2NH \qquad (C')$$

wherein $R^{1'}$ and $R^{2'}$ have the same definition as R^1 and R^2 in claim 1, with the proviso that the compound of formula (C') is not identical to the compound of formula (C) used in step (i), to give a compound of formula (I); or

a process for producing an anthraquinone of formula

(II) as defined in claim 1 in which the two groups R^3 are not the same and/or the two groups R^4 are not the same, which process comprises:

(i) reacting an intermediate of formula (A'):



(A')

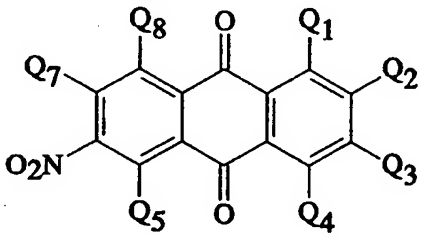
in which:

W_2 is $HNCO(CH_2)_nZ$, wherein Z is a leaving group and n is an integer of from 1 to 6, and Q_1, Q_3, Q_4, Q_5, Q_7 and Q_8 are as defined in claim 1;

with a compound of formula (D)

$$R^3R^4NH \qquad (D)$$

wherein R^3 and R^4 are as defined in claim 1, to give a compound of formula (G):

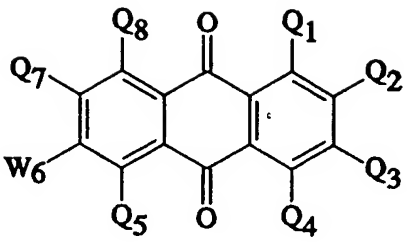


(G)

wherein Q_2 is as defined in claim 1;

(ii) converting the NO_2 group to an NH_2 group;

(iii) reating the product of step (ii) with $Z(CH_2)_nCOZ$ wherein Z is a leaving group and n is an integer of from 1 to 6 to give a product of formula (H):



(H)

in which W_6 is $HNCO(CH_2)_nZ$;

(iv) reacting the product of step (iii) with a compound of formula (D'):

$$R^{3'}R^{4'}NH \qquad (D')$$

wherein $R^{3'}$ and $R^{4'}$ have the same definition as R^3 and R^4 in claim 1, with the proviso that the compound of formula (D') is not identical to the compound of formula (D) used in step (i), to give a compound of formula (II).

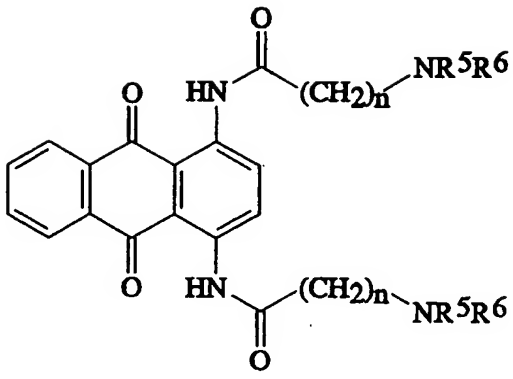
10. A process for the production of a quaternary ammonium salt of an anthraquinone of formula (I) or formula (II) according to claim 1, which process comprises treating an anthraquinone of formula (I) or (II) with an alkylating agent.

11. A compound according to claim 1 for use in the inhibition of telomerase.

12. A compound according to claim 11 for use in the treatment of cancer.

13. A pharmaceutical composition comprising a compound according to claim 1 and a pharmaceutically acceptable carrier or diluent thereof.

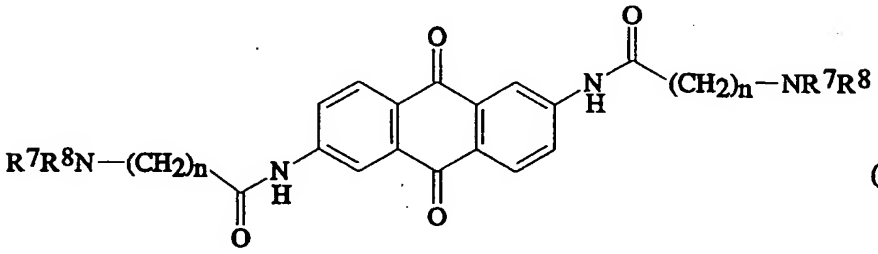
14. Use of a compound according to claim 1 or a compound of formula (III):



(III)

wherein R^5 and R^6 are each independently ethyl; or R^5 and R^6 together with the nitrogen atom to which they are attached, represent a heterocyclic group which is a 2-hydroxymethyl-1-piperidino or 1-piperidino group; and n is 2;

or a compound of formula (IV):



(IV)

in which R^7 and R^8 are each independently an ethyl or 2-hydroxyethyl group; or R^7 and R^8 together with the nitrogen atom to which they are attached represent a heterocyclic group which is a 1-piperidino, 2-hydroxymethyl-1-piperidino or 4- or 2-(2-hydroxyethyl)-1-piperidino group; and n is 1, 2 or 3;

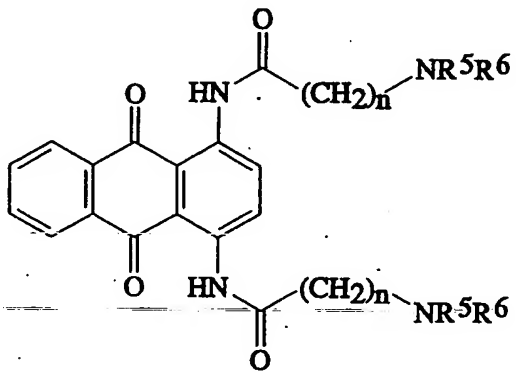
or a pharmaceutically acceptable salt thereof;
in the manufacture of a medicament for inhibiting the activity of telomerase.

15. Use according to claim 14 for the manufacture of a medicament for use in the treatment of cancer.

16. Use according to claim 14 wherein the anthraquinone of formula (III) or (IV) or salt thereof is selected from:
1,4-Bis[3-(2-hydroxymethyl-1-piperidino)propionamido]anthracene-9,10-dione;
1,4-Bis[3-(diethylamino)propionamido]anthracene-9,10-dione;
1,4-Bis[3-(2-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione;
1,4-Bis[3-(1-piperidino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(2-hydroxymethyl-1-piperidino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(2-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(4-(2-hydroxyethyl)-1-piperidino)propionamido]anthracene-9,10-dione;
2,6-Bis[3-(bis(2-hydroxyethyl)amino)propionamido]anthracene-9,10-dione;
2,6-Bis(3-(1-piperidino)propionamido)anthracene-9,10-dione diacetate;
2,6-Bis(2-(4-morpholino)acetamido)anthracene-9,10-dione diacetate;
2,6-Bis(2-diethylaminoacetamido)anthracene-9,10-dione diacetate;
2,6-Bis(3-(4-morpholino)propionamido)anthracene-9,10-dione diacetate; and
2,6-Bis(3-(4-(2-hydroxyethyl)-1-piperazino)-propionamido) anthracene-9,10-dione diacetate.

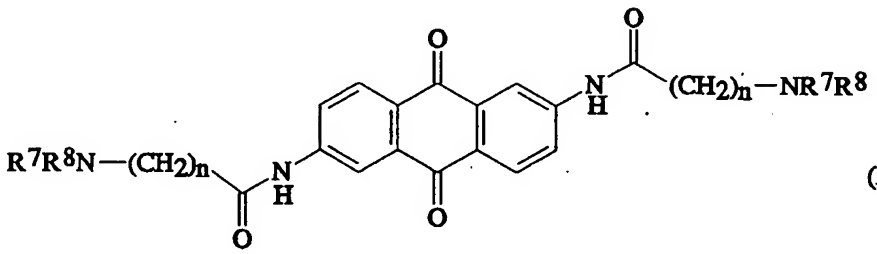
17. A method of treating a host suffering from

cancer which method comprises administering thereto a pharmaceutically effective amount of a compound of formula (I) or formula (II) as defined in claim 1 or a compound of formula (III):



(III)

where
in R^5 and R^6 are each independently ethyl; or R^5 and R^6 together with the nitrogen atom to which they are attached, represent a heterocyclic group which is a 2-hydroxymethyl-1-piperidino or 1-piperidino group; and n is 2;
or a compound of formula (IV):



(IV)

in which R^7 and R^8 are each independently an ethyl or 2-hydroxyethyl group; or R^7 and R^8 together with the nitrogen

atom to which they are attached represent a heterocyclic group which is a 1-piperidino, 2-hydroxymethyl-1-piperidino or 4- or 2-(2-hydroxyethyl)-1-piperidino group; and n is 1, 2 or 3;

or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/GB 97/03446

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C237/04 A61K31/16 C07D295/14 A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 00265 A (CANCER RESEARCH TECHNOLOGY LTD) 10 January 1991 cited in the application see claims; examples ---	1-17
A	AGBANDJE, MAVIS ET AL: "Anthracene-9,10-diones as potential anticancer agents. Synthesis, DNA-binding, and biological studies on a series of 2,6-disubstituted derivatives" J. MED. CHEM. (1992), 35(8), 1418-29 CODEN: JMCMAR;ISSN: 0022-2623, XP002063825 cited in the application see page 1419 --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 April 1998	25/05/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sánchez García, J.M.

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INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/GB 97/03446

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category ¹	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	TANIOUS, FARIAL A. ET AL: "Substituent position dictates the intercalative DNA-binding mode for anthracene-9,10-dione antitumor drugs" BIOCHEMISTRY (1992), 31(46), 11632-40 CODEN: BICHAW;ISSN: 0006-2960, XP002063826 cited in the application see page 11632	1-17
A	COLLIER, DAVID A. ET AL: "Synthesis, molecular modeling, DNA binding, and antitumor properties of some substituted amidoanthraquinones" J. MED. CHEM. (1988), 31(4), 847-57 CODEN: JMCMAR;ISSN: 0022-2623, XP002063827 cited in the application see page 847 - page 848	1-17
A	US 3 859 315 A (SANTILLI ARTHUR A ET AL) 7 January 1975 see claims	1-17
A	WO 86 00892 A (BIBER RUDOLF) 13 February 1986 see claims	1-17
A	HOFFMANN, SIEGFRIED ET AL: "Mono- and bis-basic anthraquinones" Z. CHEM. (1986), 26(6), 206-7 CODEN: ZECEAL;ISSN: 0044-2402, XP002063828 see page 206	1-17
A	WINKELMANN, E. ET AL: "Chemotherapeutically active anthraquinones. I. Aminoanthraquinones" ARZNEIM.-FORSCH. (1979), 29(10), 1504-9 CODEN: ARZNAD;ISSN: 0004-4172, XP002063829 see page 1505 - page 1507	1-17
A	MARTELLI, SANTE ET AL: "Synthesis and antineoplastic evaluations of 1,4-bis(aminoalkanamido)-9,10-anthracenediones" J. MED. CHEM. (1988), 31(10), 1956-9 CODEN: JMCMAR;ISSN: 0022-2623, XP002063830 see page 1956 - page 1957	1-17
A	GATTO, BARBARA ET AL: "Peptidyl Anthraquinones as Potential Antineoplastic Drugs: Synthesis, DNA Binding, Redox Cycling, and Biological Activity" J. MED. CHEM. (1996), 39(16), 3114-3122 CODEN: JMCMAR;ISSN: 0022-2623, XP002063831 see page 3115	1-17

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/GB 97/03446

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9100265 A	10-01-1991	EP 0482119 A	29-04-1992
US 3859315 A	07-01-1975	NONE	
WO 8600892 A	13-02-1986	AU 4679985 A	25-02-1986
		EP 0191058 A	20-08-1986
		JP 61502891 T	11-12-1986
		US 4794125 A	27-12-1988